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ADRENAL CORTICAL VIRILISM[1]

F. D. W. LUKENS AND H. D. PALMER

From the George S. Cox Medical Research Institute, University of Pennsylvania, and the Institute of the Pennsylvania Hospital

PHILADELPHIA, PENNSYLVANIA

FOR MANY YEARS it has been known that adrenal cortical tumors might be associated with disturbances of the secondary sexual characteristics, and more recently it has been noted that these tumors vary in the number and nature of the symptoms they produce. Thus, some adenomas of the adrenal cortex have appeared as incidental findings at autopsy; others are associated with virilism alone; and still others appear as the only gross lesion in the extensive metabolic disturbance of Cushing's syndrome (1). Young has summarized the literature in his text (2).

The patient described is a girl with virilism in whom (*a*), early surgical exploration revealed no tumor of the ovaries or adrenals. (*b*), Subsequently an adrenal tumor was diagnosed and was removed with striking symptomatic improvement. (*c*), Before the tumor was removed her urinary androgen excretion was greatly elevated and estrogens were very high by one method of assay, but were normal by another method. The results of the estrogen studies are significant in view of the variation in the estrogen excretion of similar patients reported in the literature. (*d*), In spite of the bodily virilism she was psychologically feminine at all times. The surgical features of this case have already been reported (3).

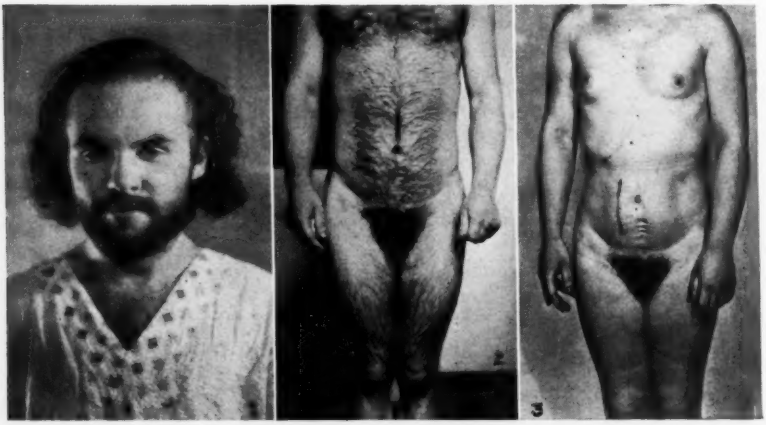
History. M.H., a white girl, was well until 1926 when at the age of 6 she had scarlet fever. A few months after this her parents noticed that her voice was becoming deep and hoarse. Six months later hair appeared on her upper lip, and by the time she was 9 years old she had a full beard and her entire body was covered with hair. She had a masculine figure, great muscular strength, and at the age of 8 to 10 was considered quite tall.

Her psychological outlook remained distinctly feminine throughout the course of her condition; she played with other girls, preferred dolls, enjoyed girlish games, learned to sew and knit, helped with household work and later showed great interest in babies. She spoke of having boy friends some day and hoped eventually to marry and have children of her own. Psychometric studies at the age of 13 gave results slightly below normal (Stanford Binet I.Q., 80; mental age 9 yr. and 9 mo.). This may be partially explained by irregular attendance at school.

As she grew older the absence of menstruation, lack of development of the breasts, and increasing mental distress concerning her hairiness were added to the picture. The family, social and past medical histories were not significant.

[1] Aided by a grant from the John and Mary R. Markle Foundation.

She was first seen at the Neurological Institute (H.D.P.) in 1931, when, at the age of 11, an exploratory laparotomy was performed which revealed normal female pelvic organs, except that possibly the uterus was larger than that of the average child of her age. Both adrenal regions were palpated through the abdominal incision and no gross pathology could be detected. Her visual fields and roentgenogram findings of the pituitary fossa were normal.



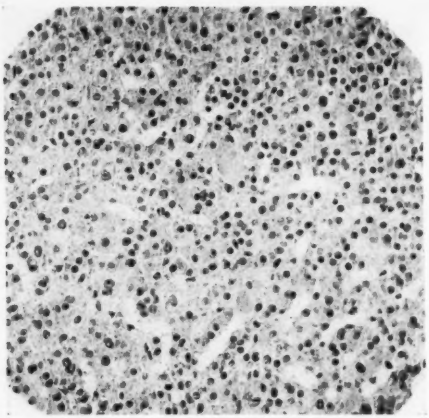


Fig. 1. PREOPERATIVE APPEARANCE WITH ARMS SHAVED. Fig. 2. PREOPERATIVE APPEARANCE, UNSHAVED. Fig. 3. TWO YEARS AND 10 MONTHS AFTER REMOVAL OF TUMOR. Fig. 4. ADRENAL CORTICAL TUMOR (×160).

In March 1936 she was first admitted to the University of Pennsylvania Hospital when physical examination showed extreme hirsutism of her face and body. Acne was present on the face and back. She was short, not obese, with relatively wide shoulders, narrow hips, a deep voice and strictly masculine or prepuberal breasts. The hair on the scalp was coarse, wiry and thin. The clitoris was enlarged. Her blood pressure of 110/80 was in agreement with many previous observations. Numerous blood count and urinalysis findings were normal. The Wassermann test result was negative.

The premature union of the epiphyses reported in virilism was present in this patient. In March 1936, at the age of 16, it was noted that there was complete union of the epiphyses of the pelvic bones, upper ends of the femora and of the vertebral spines. The roentgenolo-

TABLE 1

Date	Age	Ht.	Wt.	B.M.R.	Serum Ca
	yr.	in.	lb.		mg./100 cc.
Preoperative					
Sept. 1931	11	58	106	−15	11
April 1933	13	59	111	−9	
March 1936	16		106		
Feb. 1937	17	59	106		
Postoperative					
June 1937	17			+2	
May 1938	18	59	99	−1	11.4
May 1939	19	59	100	−12	

gist reviewing these films expressed the opinion that at 16 she had the bone age of 24 to 26 years. There was no decalcification of the bones. Although no roentgenograms of suitable bones were taken at an earlier age, she had only grown an inch since the age of eleven. In table 1 her age, height and weight are recorded for the period of observation and the data suggest that epiphyseal union may have occurred as early as 11 years of age. In this connection two normal serum calcium levels are recorded, and the metabolic rates show that no thyroid disturbance was found to account for the premature union.

In table 2 the results of several glucose tolerance tests are summarized. Abnormally high curves (low sugar tolerance) have been found in patients with adrenal tumors (1, 4) and the height and prolongation of the curves suggest impaired carbohydrate metabolism in this patient. However, she has not been regarded as diabetic because the urine was always negative for sugar and additional fasting and post prandial blood sugars have been normal.

At this time a roentgenogram after air injection of the adrenal areas (5) revealed a large tumor in the region of the right adrenal. The left adrenal was not well visualized. A urogram showed a functioning urinary tract with no distortion of the kidney outlines. As a further precaution, the cholecystogram indicated that the gallbladder functioned normally and was distinct from the mass on the right. Operation was advised but was refused at this time, and she returned to the care of the Neurological Institute.

The patient was readmitted to the University of Pennsylvania Hospital on February 4, 1937 for operation. During the year there had been no change in her appearance. A roentgenogram after air injection of the adrenal areas again showed the tumor on the right side. Operation was performed on February 8, 1937 by Dr. E. L. Eliason, who removed the tumor through a right subcostal incision. The left adrenal could not be felt at operation. A reaction similar to thyroid crisis was a feature of the postoperative period (3) which ended in complete surgical recovery.

Her appearance before the excision of the tumor is shown in figures 1 and 2; figure 3 illustrates the changes in hair distribution and bodily contour after this operation.

The tumor was well encapsulated, and measured 6 cm. in diameter. On section it was reddish brown and mottled in color. Microscopic section (fig. 4) showed a very cellular structure with little stroma. The cells were of the adrenal cortical type and only a small amount of lipoid was present.

TABLE 2. GLUCOSE TOLERANCE TESTS

Date	Blood glucose						Glucose given
	Fasting	½ hr.	1 hr.	2 hr.	3 hr.	4 hr.	
	mg./100 cc.	mg./100 cc.	mg./100 cc.	mg./100 cc.	mg./100 cc.	mg./100 cc.	gm.
Sept. 30, 1931	96	156	178	116			86
Feb. 13, 1936	85	168	195	164	158	95	—
Feb. 5, 1937	58	105	75	50			90
June 15, 1937	87	140	136	155	138		76
May 19, 1938	85	145	108	98	105	80	79

Following operation she has improved steadily during the 3 years since the excision of the tumor. The rate at which the various findings were altered after operation (Feb. 8, 1937) may be seen in the following summary. At 3 weeks the voice was thought to be less husky, and at present (3 years later) it is softer but is still a deep feminine voice. The disappearance of acne in the first 3 weeks was a striking change, which has persisted. At 3 months she had her first normal menstruation and menstrual periods have been regular ever since. After operation she complained of some weakness and when convalescence was complete it was apparent that this was normal feminine strength regarded as weakness in comparison to her masculine muscle tone before operation.

At one year the hair of the head was softer and finer in texture but there was no growth of new hair in the thin areas. There was no change in the scalp in the second year. The beard receded from the eyes somewhat and the skin of the face became distinctly softer. There was very little change during the second year, although the rate of growth of the

TABLE 3. URINARY HORMONE ASSAYS

Dates of collection (inclusive)	Androgens, I.U./day	Estrogens, M.U./day		Menses[1]
		Frank method (8)	Gallagher et al. method (5)	
		Before operation		
Feb. 4–7, 1937	160	4580	35	Amenorrhea
		After operation		
Feb. 22–27, 1937		10		Amenorrhea
March 9–13, 1937	6		12	May 1, 1937[2]
June 15–20, 1937	12		70	
Jan. 30, 1938		190		Jan. 3, 1938
Jan. 31, 1938		240		
May 20, 1938		144		
May 21, 1938		133		June 1, 1938
May 10–12, 1939		7		
May 13–15, 1939		6		May 16, 1939

[1] Dates of onset of menses preceding or following collections.
[2] First normal menses; regular to present time.

beard has decreased. This persistence of the beard, in spite of improvement in other respects, has been discouraging. The bodily hair became finer within 3 months and at one year had largely disappeared. The improvement was most prompt over the thorax, but much hair remained on the lower abdomen and back at this time. During the second year the hair on the lower body also decreased, so that at present she has normal feminine pubic hair and no excess of body hair. The hair on her arms and legs became pale and fine, and although it was within the normal range for a brunette at the end of one year, improvement continued so that she now has almost no hair on her extremities.

At three months she began to have puberal symptoms in her breasts and at 2 years normal adult breasts had developed. The general bodily contour changed from clearly visible masculine muscles to a smooth feminine outline of the limbs and slight enlargement of the hips. On August, 1939, she developed intestinal obstruction for which operation with lysis of adhesions was performed. The adhesions were not at the site of the adrenal operation and their origin was not established. She made an uneventful recovery from this and when last heard from 3 years after her adrenal operation was in good health.

Table 3 shows the male and female sex hormone assays of her urine. The male hormone assays were conducted by Dr. R. I. Dorfman. The methods used and the results obtained in other patients with adrenal tumors have been presented (6, 7, 8). Estrin assays were made by Dr. Franklin Payne by Frank's method (9) and by Dr. Dorfman (6), both using spayed mice as the test animal. The results will be considered in the discussion.

DISCUSSION

The patient described developed virilism at the age of 6 and was greatly improved

after the removal of an adrenal cortical adenoma 11 years later when she was 17 years old. The result was excellent except for the beard which has only regressed slightly. The persistence of this girl's beard is comparable to the persistence of the beard after castration in the adult male. In both cases regression of the beard is very slight although the organ associated with its development has been removed.

The difficulty of diagnosis by even surgical palpation is illustrated by the failure to find a tumor in 1931 and by the surgeon's note that in 1937 the left adrenal was not palpable at operation. The aid of the air injection roentgenogram was noteworthy. It is possible that in 1931 this girl had adrenal cortical hyperplasia which was the precursor of the adenoma found in 1937. The sequence of hyperplasia→adenoma, which has been studied in the case of the thyroid, would serve to explain in part the difficulties of early diagnosis in such patients.

The diminution in urinary androgen excretion after operation agrees with the findings of others (7). As far as the authors are aware this is the first patient to have estrogen assays by two different methods and the difference in the results is striking. The Frank method yielded a very high value for estrogens preoperatively, which agrees with the reports on certain cases of adrenal tumors (10, 11). On the other hand Walters and Kepler (12) have found no such high estrogen values in their series of adrenal tumors. Our experience indicates that these discrepancies may be due to the different methods used in extracting the urine for assay. Frank's method extracts androgens and estrogens together while the method of Gallagher et al. (6) separates these substances before assay. Since the androgens have some estrogenic power their separation is necessary in this type of patient. Whether the particular androgens excreted by this patient were more estrogenic than usual cannot be stated, but the high estrogen assay by Frank's method is to be noted in connection with the normal feminine psychological pattern of this patient during her years of virilism. One can only speculate on the rôles played by education and hormones in the mental state under these unusual circumstances.

SUMMARY

A girl with virilism of 11 years duration was greatly improved after the removal of an adrenal cortical adenoma. Her body hair and acne disappeared, her breasts developed, and menstruation began. The urinary excretion of androgens was increased before and became normal after operation. Urinary estrogen excretion was determined preoperatively by two methods with widely different results which may explain the findings of others in these cases. In our patient, a feminine psychological pattern was observed throughout the period of masculinization.

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ESTROGENIC EFFECTS UPON TUBAL CONTRACTILITY AND THE VAGINAL SECRETION IN THE MENOPAUSE

STUDY OF 24 CASES WITH THE AID OF UTEROTUBAL INSUFFLATION[1]

PHINEAS BERNSTEIN AND MORRIS FERESTEN

From the Mt. Sinai Hospital[2]

NEW YORK CITY

IN A PAPER read before the American Gynecological Society, May 1932, Rubin (1) reported his experience with insufflation in 7 women of the menopause age. There were no tubal contractions in 3 instances, while in 4 of the 7 cases the contractions were infrequent and barely reached an amplitude over 5 mm. Hg. He also considered the possible influence of ovarian hormonal function on the fallopian tubes in relation to the different phases of the ovarian cycle. From comparative observations of many graphs obtained during clinical tubal insufflation, a definite impression was gained that there was a possible relationship between tubal contractions and the ovulation menstruation cycle.

This impression gained from clinical experience with the test for tubal patency led Rubin (2, 3) to suggest in 1927 and in 1928 that parallel investigations be "carried out in estimating the quantity of female sex hormone." Along the same line of investigation, Morse and Rubin (4) applied the method of uterotubal insufflation in the *macacus rhesus* with the object of assaying pharmacologic and hormonal effects on tubal and uterine contractions.

The effect of estrogen (progynon-B) on tubal contractions in secondary amenorrhea was studied by Rubin (5), February 1937, in a woman 31 years old. The number of cases studied from this point of view appeared to be too small to warrant definite conclusions, but were sufficiently suggestive to spur us on to further study.

Observations upon the vaginal secretion in the menopause were reported by Papanicolau and Shorr (6). They found a specific cytological pattern of regression. Geist, Salmon and Mintz (7) made use of the vaginal smear as a check upon the tubal contractility observed in the menopause. They state that "coincident with the impairment in tubal contractility, the vaginal smears exhibit signs of various degrees of estrogenic hormone deficiency." They also state that the administration of estrogenic hormone produced restoration of tubal contractions as well as full estrogenic effects upon the vaginal secretion.

We have continued the study of this relationship between estrogenic substances, tubal contractions, and the vaginal secretion, on a larger series of cases in order: *a*), to arrive at an average graphic pattern for the type of tubal contractions commonly present in the menopause; *b*), to determine to what extent the vaginal smear reflects the atrophic changes said to occur in the menopause; *c*), to note whether there is a uniform correlation between tubal contractions and the vaginal smear and *d*), to ob-

[1] Read at the Fortnight Review of Endocrinology of the N. Y. Academy of Medicine, November 27, 1939.

[2] And from the Gynecological Service of Dr. I. C. Rubin to whom we are indebted for the suggestion and supervision of this study.

serve the effect of estrogenic therapy upon the vaginal secretion and tubal function.

Twenty-four patients in the menopause, both natural and after x-ray castration, were studied. Their ages varied from 38 to 55 years. In the majority, the duration of menopause was more than one year; the minimum period was 4 months and the maximum 8 years.

METHODS

The Rubin insufflation kymographic apparatus (8) was used for the graphic recording of the tubal contractions.

Carbon dioxide gas under 15 pounds of pressure was introduced into the uterus and tubes through a cannula in the cervix. Tubal contractions manifested themselves by oscillations on the mercury manometer and were concomitantly recorded upon the kymograph. In order to minimize the personal error all insufflations were performed by one of us (M. F.), and the same apparatus was used throughout for all tests.

Each patient was insufflated 4 times at intervals of 2 to 5 days. Estradiol benzoate, i.e., dimenformon benzoate (Roche-Organon) or progynon-B (Schering),[3] was then injected intramuscularly. The usual single dose, given at intervals of from 1 to 7 days, varied from 2000 to 50,000 R.U.; each patient receiving a varying total of 8000 to 220,000 R.U. Within 24 hours of the estrogenic therapy a second series of tubal graphs were started, 4 insufflations being made at intervals of 2 to 5 days.

The insufflation records obtained before and after estrogenic therapy were then compared in respect to the following 4 factors.

A. Initial Rise Pressure, representing the gas pressure in millimeters of mercury required to overcome the resistance of the uterotubal junction.

B. Range Tone Pressure, or the average level in millimeters of mercury at which tubal contractions are maintained above the base line.

C. Amplitude, height of each contraction in millimeters of mercury.

D. Rate, frequency of contractions per minute.

The actual measurement of these factors in each graph enabled us to arrive at an average value for tubal function in the menopause, and to determine the effect of estrogenic substances on tubal activity.

Vaginal smears made just preceding each insufflation were fixed in methyl alcohol and stained with aqueous fuchsin. They were then classified into the negative or atrophic phase (low estrogen level), and the positive or follicle phase (high estrogen level).

RESULTS

The average menopause graph. A typical pattern of tubal function in the menopause was obtained by averaging all measurements for each component factor in the, 80 kymographic records obtained from the 24 patients comprising this study (fig. 1). These average values were as follows: initial rise pressure, 56.97 mm. Hg; range tone 37.40 mm. Hg; contraction rate, 2.99 per minute, and amplitude of contractions, 4.72 mm. Hg.

In over half of the patients studied, the graph factor values were well below those computed for the average menopause graph. Thus, in 14 of the 24 patients (58.3%), the initial rise pressure varied from 31.25 to 55 mm. Hg; in 17 patients (70.9)%, the range tone varied from 18.8 to 36 mm. Hg; in 13 patients (54.2%), the amplitude or height of each contraction varied from 1.36 to 4.40 mm. Hg., and in 12 patients (50%), the contraction rate varied from 0.66 to 4.6 per minute.

[3] We are grateful to Roche-Organon and Schering Corp. for generous supplies of dimenformon benzoate and progynon-B.

Rubin has noted that in the normally menstruating woman, the initial rise pressure usually varies between 60 and 100 mm. Hg, the contraction rate is between 4 and 6 per minute, and the amplitude of contractions varies from 15 to 50 mm. Hg. In this series of menopause women, 15 out of 24 (62.5%) had an initial rise pressure below 60 mm. Hg; 20 patients (83.4%) had less than 4 contractions per minute, and in seventeen patients (71%) the amplitude of contractions was less than 5 mm. Hg. Thus, in more than 62.5% of the menopause patients studied here, values were ob-

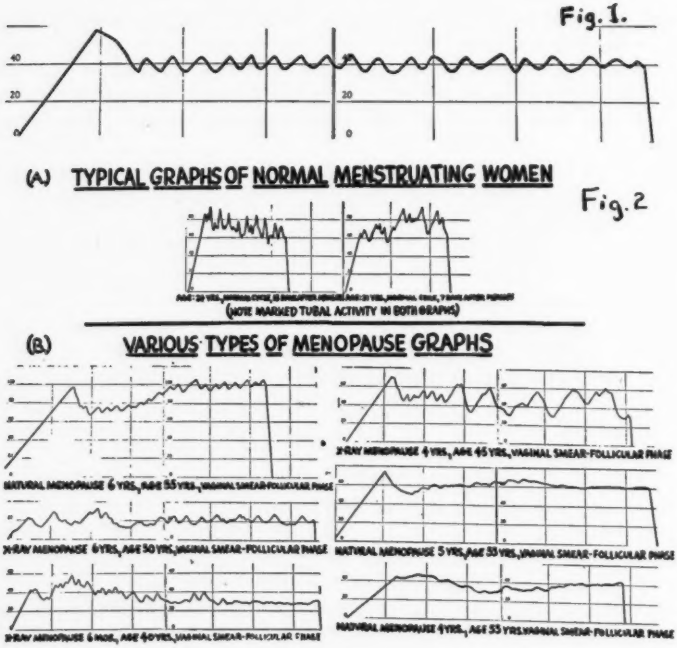

Fig. 1. THE AVERAGE MENOPAUSE GRAPH, a composite curve representing the average values obtained by exact measurements of 80 insufflation records in 24 menopausal women. Fig. 2. COMMON TYPES OF GRAPHS obtained during the menopause indicating lowered tubal activity, as compared to typical graphs of normally menstruating women. The vaginal secretion of all these 6 climacteric patients showed follicular characteristics.

tained indicating tubal function lower than that found by Rubin for the average normally menstruating woman. In a minority of our climacteric patients, however, tubal function simulating that of the normal woman with good ovarian function was seen (fig. 2).

Vaginal secretion in the menopause. The vaginal secretion of 19 of the 24 patients was studied. Thirteen of the 19 menopause women (68.4%) gave vaginal smears with follicular characteristics, as found in normally menstruating women. The 6 remaining cases (31.6%) had smears with the atrophic pattern, suggesting marked estrogen deficiency. This is in accord with the report of Salmon and Frank (9) that "in only about 40% of castrates and post-menopause cases (45 women) do we find uniformly negative smears." Rothermich (10), too, stated that in nearly half of his

Fig. 3. INCREASED TUBAL FUNCTION IN MENOPAUSE AFTER 40,000 R.U. DIMENFORMON BENZOATE (ROCHE-ORGANON). The initial rise pressure and range tone were increased 40 mm. Hg each, the contraction rate increased 0.7 per minute, and the amplitude showed a slight decrease of 0.3 mm. Hg. Both before and after estrogenic therapy, the vaginal smears showed follicular characteristics. Fig. 4. INCREASED TUBAL FUNCTION IN MENOPAUSE AFTER 40,000 R.U. DIMENFORMON BENZOATE (ROCHE-ORGANON). The initial rise pressure increased 33.0 mm. Hg., the range tone increased 18.25 mm. Hg, the amplitude increased 1.98 mm. Hg, and there were 1.09 additional contractions per minute. All smears advanced from the atrophic to the follicular pattern after treatment.

menopause patients, vaginal smears with follicular characteristics were observed. These findings are also consistent with the bioassay studies of Frank et al. (11) in which they observed that the estrogenic factor continues to be excreted after surgical removal of the ovaries as well as after physiological menopause and x-ray castration.

One would expect, in the 6 untreated menopause patients, showing estrogenic deficiency in their vaginal smears (atrophic pattern), a corresponding lowering of tubal function, and in the 13 remaining cases, showing follicular vaginal smears, a corresponding higher tubal activity. In our analysis, we were unable to demonstrate this correlation with consistency (fig. 2, 3). Thus, only 2 of the 13 patients with follicular smears, showed good tubal function; in 4 instances tubal function was very

poor, and in the remaining 7 only fair. Also, 4 of the 6 cases with atrophic smears, showed good tubal function; only 2 of these patients had poor tubal activity.

Effect of estrogenic therapy on tubal function in the menopause. In studying the effect of estrogens on tubal function, we found the action of dimenformon benzoate and progynon-B to be identical, so that no differentiation between these two drugs

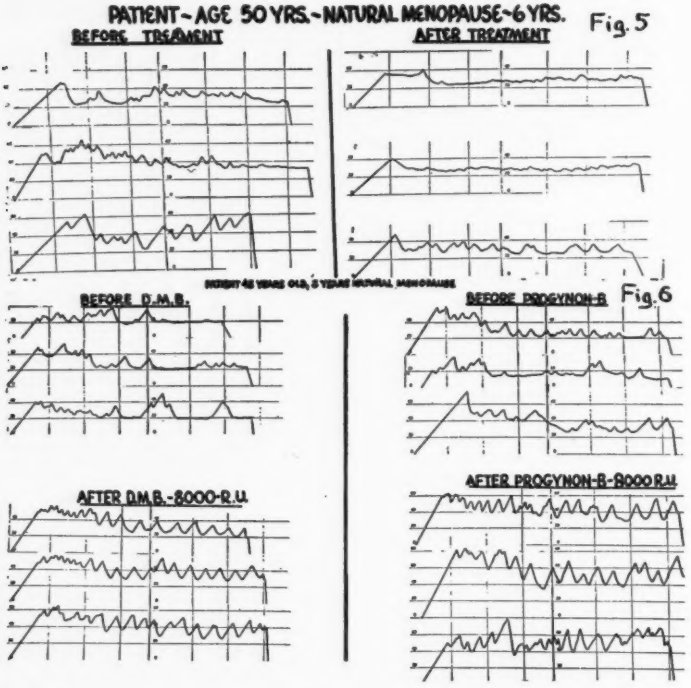

Fig. 5. After estrogenic therapy, there was a decrease in the initial rise pressure of 43.3 mm. Hg, and in the range tone of 5.4 mm. Hg. There was 0.14 of a contraction less per minute with a decrease in amplitude of 2.04 mm. Hg. This is one of 4 cases out of 7, showing a decrease and suggesting an inhibition of tubal activity after large doses of estrogen (120,000 to 220,000 rat units). Fig. 6. Comparison of dimenformon benzoate and progynon-B in same patient. After treatment, tubal function was increased with both drugs as follows.

	Initial rise	Range tone	Frequency	Amplitude
Dimenformon benzoate	+4.3	+ 8.75	−.20	+6.18
Progynon-B	+15	+10.25	+.30	+3.1

Vaginal smears before treatment showed atrophic characteristics. After estrogenic therapy the follicular type of smears were present.

will be made. For analysis, we divided our patients into 2 groups, according to the dosage of hormones obtained. One group of 15 patients received from 8000 to 70,000 R.U. of estradiol benzoate; the other group of 9 patients received from 120,000 to 220,000 R.U.

Initial rise pressure and range tone. The initial rise pressure was found to be increased from 4 to 40 mm.Hg, in all of the 15 patients who received from 8000 to 70,000 R.U. Four of the 9 patients receiving from 120,000 to 220,000 R.U. also showed

an increase of from 1.8 to 21.3 mm. Hg. The remaining 5 cases showed a decrease of from 2.0 to 66.5 mm. Hg.

Similarly, the range tone pressure was elevated from 0.5 to 40 mm. Hg, in all the cases which received from 8000 to 70,000 R.U.; 5 of the patients receiving the larger doses also showed an increase of from 3.75 to 12 mm. Hg. Four patients who received the larger doses, showed a decrease in range tone. Significant is the fact that a decrease in the initial rise and range tone pressures occurred only following the larger doses of estrogen.

The above findings show that there is a definite increase in uterotubal tone (initial pressure and range tone) following the use of estrogens in moderate doses (fig. 3, 4). When large doses are employed (120,000 to 220,000 R.U.) a definite decrease in uterotubal tone frequently ensued (fig. 5).

Contraction rate. There were from 0.2 to 2.68 additional contractions per minute in 11 of the 15 patients receiving the smaller amounts of estrogenic substances; in 4 instances there were from 0.2 to 1 contraction less per minute.

Seven of the 9 patients receiving large doses had 0.3 to 5.14 additional contractions per minute; in two instances there was a decrease of 0.14 and 0.17 of a contraction per minute. In all, 75% of the menopause patients showed an increase in the contraction rate following estrogenic therapy.

Amplitude of contractions. There was an increase in the height of contractions of 0.42 to 6.8 mm. Hg, in 10 of the 16 patients who received from 8000 to 70,000 R.U. of estrogen; in the remainder there was a diminution of 0.09 to 3.99 mm. Hg.

An increase in the height of contractions was noted in 5 of the 9 patients receiving the larger doses, of from 0.6 to 1.60 mm. Hg. In the remaining 4 instances, there was a decrease in amplitude, ranging from 0.61 to 2.02 mm. Hg.

The height of tubal contractions was increased, therefore, in 15 of the 24 menopause patients, as a result of estrogenic therapy.

In summarizing the effect of estrogens on each of the constituent graph factors, it is of interest to note that the initial rise pressure was advanced in 79.2% of the 24 patients; the range tone was higher in 83.3%; the contraction rate was increased in 75%; and the amplitude of contractions showed an increase in 62.5%. These findings point to a definite stimulating effect in the majority of the 24 cases under study.

Four patients who were originally treated with dimenformon benzoate, after an interval of 4 months, were again studied after equal doses of progynon-B. The results were strikingly similar (fig. 6).

The effect of estrogenic therapy on the vaginal secretion. In all 6 cases showing an initial atrophic smear pattern, the administration of estrogenic hormones resulted in an advancement to the follicular phase. The 13 women who had a follicular type of vaginal smear before therapy showed no changes in the vaginal secretion after receiving estrogenic substances.

Despite the positive change from atrophic to follicular phase in the vaginal smear of 6 patients after estrogenic therapy, only 3 showed a corresponding increase of tubal function. In one of the remaining 3 cases there was actually a decrease in function, while in the other 2 there was no change.

The age of the patients, the duration of menopause and the type of menopause (natural or x-ray) appeared to bear no relationship to the results obtained in this study.

SUMMARY

Tubal contractions of 24 menopause women, as determined by uterotubal CO_2 kymographic insufflation, were studied before and after estrogenic therapy. The pres-

sure changes were computed metrically. The vaginal secretion was also studied and compared in 19 cases.

A composite graph, suggesting an average value for tubal function in the menopause was obtained by analysis of the records of 80 control insufflations. In more than one-half of the untreated menopause patients, studied here, the tubal function figures were well below those of the composite graph averages. In at least 62.5% of the climacteric patients, tubal function was lower than that found by Rubin in normally menstruating women.

In 13 of 19 menopause patients (68.4%), all vaginal smears showed follicular characteristics (high estrogen levels). In the 6 remaining patients (31.6%) we found the atrophic pattern (low estrogen levels).

In the untreated menopause patients there was no constant correlation between the type of vaginal smear and tubal function; smears showing the follicular phase were often associated with poor tubal function.

Estrogenic therapy increased uterotubal tone (initial rise pressure and range tone) in over 80% of the 24 menopause cases. Tubal contractions were increased, as evidenced by a higher contraction rate in 75% of the patients and by an increase in the amplitude in 62.5% of the patients.

In all 6 cases showing the atrophic vaginal pattern (low estrogen phase), the vaginal smear picture was advanced after estrogenic therapy to the follicular phase (high estrogen level). In 3 cases there was a corresponding advance in tubal function, while in the remaining 3 instances there was no correlated action.

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TESTOSTERONE PROPIONATE AND THE MALE CLIMACTERIC[1]

H. B. THOMAS AND R. T. HILL

From the Department of Anatomy, Indiana University School of Medicine
BLOOMINGTON, INDIANA

THE QUESTION of the male climacteric has long been of interest. For many men such a period does not become manifest, while in others it appears as absence of libido or as a depressive melancholia. That men have involutional melancholia can be confirmed by psychiatrists, for most institutions caring for mental cases have had these patients.

Werner (1) gives a very adequate description of the male climacteric, in which he states: "The climacteric disturbance may be so severe in some men that they become despondent and develop a psychosis with thoughts of self-destruction. It is very probable that many men have committed suicide and no one could understand the reason for their having done so."

The term "male climacteric" is being widely used at present, both in the clinic and the experimental laboratory. Because of such usage we here subscribe to the term, but in a limited way. The cases described were diagnosed as involutional melancholia, thought to be caused by a lack of sufficient male hormone elaboration in the individual for mental stability. In that sense we use the term 'male climacteric.'

Although androgenic substances have been administered to human males with the idea of increasing libido, penis size, erections and the act of mating, little has been done relative to the condition of melancholia. Werner (1) reports 2 cases which were treated and good results obtained, but which lasted for only a short period beyond the duration of the treatment. Hamilton (2) reports decreased headaches and cessation of hot flushes by the use of male hormone treatment in a case of hypogonadism in a young mature male. All of these conditions returned after treatment ceased. One author (3) has raised a warning against the use of synthetic androgens in prepubertal boys, as abnormal acceleration of puberty may result. No work was done, however, concerning the climacteric. Malitch (4), while using androgens to treat acne in some selected young males, found no direct effect on the condition of the acne, and likewise the incidence of erections and nocturnal emissions was not changed.

Six cases (5) treated with testosterone propionate are recorded as follows: two of psychic impotence, two castrates, two hypogonadism, all with fair results. All of these cases were associated with other organic disturbances. They are interesting, but appear to fall into a different class than those here described.

We have treated 2 cases, diagnosed as involutional melancholia, with testosterone propionate, and the results were of such nature as to warrant presentation here.

Case 1. A white male, age 56, married, was the father of several children. His complaint was nervousness, insomnia, occipitocervical headache and lapse of memory. These characters had been present for about one year at admission. His change in emotions began with mental confusion and the belief that he was being persecuted. Until the onset he had been

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[1] The testosterone propionate was very kindly furnished by the Schering Corporation, through the courtesy of Dr. William H. Stoner.

successful in business, but it became necessary to relieve him of responsibility with progression of the symptoms. Other physical findings were negative, and the family history was unimportant. He was given 10 mg. testosterone propionate 3 times weekly for 4 weeks. The treatment was then changed to 10 mg. twice each week for a period of 6 weeks, at which time he was discharged in good health and of stable mind. At the time of this writing (7 months after discharge) the patient is still normal.

Case 2. A white male, age 48, was a farmer with a small business as a side line. This man was brought in by his wife, who stated that he had suffered a loss of memory during the past six months. This was correlated with an increasing emotional instability and insomnia. He worried continually about his business and suffered from ideas of persecution. He would wander from home, and it became necessary to watch him at all times. Physical examination findings were negative and family history was unimportant.

The patient was given 10 mg. of testosterone propionate once weekly for a period of 8 weeks. He showed a most remarkable improvement during the course of the injections, and treatment was discontinued at the end of this period because of unavoidable circumstances. After discharge, improvement in the patient's condition was steady and at this writing (10 months after beginning of treatment) the patient is normal and is managing both his farm and his business.

SUMMARY

The two cases presented here diagnosed as male climacteric (involutional melancholia), have been treated with moderate amounts of a synthetic androgen (testosterone propionate) for relatively short periods of time. Both cases made remarkable improvement during treatment, and such improvement has remained for a very definite period of time following the cessation of treatment (seven and ten months). These results are very satisfactory, and warrant the study of more cases with such treatment when the causative factor is the male climacteric.

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MENSTRUATION AND THE ENDOMETRIUM

LEO WILSON AND RAPHAEL KURZROK

From the Department of Obstetrics and Gynecology, the College of Physicians and Surgeons, Columbia University and the Vanderbilt Clinic

NEW YORK CITY

SINCE HITSCHMANN AND ADLER (1) first described the cyclic changes in the endometrial glands and Schroeder (2) correlated these changes with corresponding rhythmic phenomena in the ovaries, many investigators have searched the endometrium for an explanation of the mechanism of menstruation. The introduction of the suction biopsy (3) in 1932 has made it a simple matter to obtain human endometrium at frequent intervals. Despite this aid the exact mechanism of menstruation remains unknown. While the condition of the endometrium is a fairly reliable index of ovarian function, it does not indicate if or when menstruation will occur (4). The purpose of this paper is to report two unusual cases which we believe cast additional light on this problem.

It is generally believed that menstruation occurs as a result of degenerative changes in the endometrium following regression of the corpus luteum (5). In the normal ovulatory cycle, the secretory endometrium as a rule is cast off more or less completely during the first or second days of the flow. From then on until a corpus luteum is formed, the endometrium is in the proliferative phase. Rarely, isolated areas may be seen in which apparently previously secreting glands are undergoing involutional changes during the proliferative stage (6). This report deals with two cases in which a secretory endometrium was present after the completion of the flow, on the sixth day of the cycle.

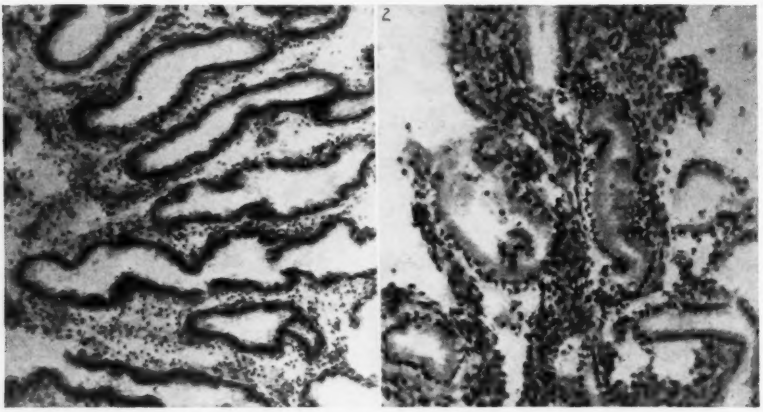
CASE REPORTS

Case 1. A. H. A white female, aged 39, grav. 1, para 0, was admitted to the Vanderbilt Clinic 9/3/35 complaining of headaches and scanty menstruation. Menses began at 15 years, occurred approximately every 28 days with a moderate flow of 3 to 4 days duration unaccompanied by pain. During the last 10 years, however, the flow became scanty, consisting of spotting for one-half hour followed by a brownish vaginal discharge of 3 days duration.

Physical examination findings were essentially negative. The basal metabolic rate was −19%. Urine assay demonstrated the presence of 8 R.U. estrogenic hormone per liter (normal); gonadotropic hormone was absent. Skull roentgenogram showed a normal sella turcica.

The patient had a regular menstrual period 10/24 to 26/35 with the usual scanty flow. On 10/29/35, which was the sixth day of the cycle, an endometrial biopsy revealed a typical secretory endometrium with large convoluted glands and edematous stroma (fig. 1). Four strips of tissue from different parts of the uterine cavity showed a fairly uniform picture. Another biopsy on the thirteenth day of the cycle (11/5/35) revealed a typical proliferative endometrium composed of small glands lined by pseudo-stratified epithelium and a dense stroma. Thus the secretory endometrium survived the menstrual flow, and the subsequent transformation to a proliferative endometrium was not associated with further bleeding. Whether the secretory endometrium was cast off before being replaced or whether it became proliferative by involution was not determined.

Case 2. E. J. A negress, aged 33, grav. 0, was admitted to the Vanderbilt Clinic complaining of post-menstrual abdominal cramps. Menses began at 14 years, occurred regularly every



SECRETORY ENDOMETRIUM ON THE 6TH DAY OF THE CYCLE. Fig. 1. *Case 1.* Fig. 2. *Case 2.*

28 days with a scant 4-day flow with mild lower abdominal cramps on the first day. During the past year the pain has been more intense and began when the flow ceased. It lasted three to four days. Physical examination revealed no significant findings.

The last regular period was 5/27 to 30/39. The next period occurred a few days earlier than usual on 6/21 to 25/39. Uterine motility studies by the intrauterine balloon method (7) on the second day of the flow revealed the large prolonged waves (fig. 3) usually seen at this stage of the ovulatory cycle. On 6/26/39, the sixth day of the cycle, when bleeding had ceased 24 hours previously, the contractions were still large and painful (fig. 4). Usually the contractions are somewhat smaller at this time. Biopsy now revealed a secretory endometrium in which secretory activity was still evident (fig. 2). In this case again the menstrual flow had run its course before the endometrial and myometrial cycles were completed.

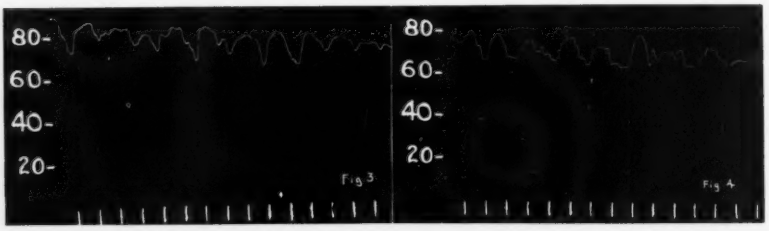


Fig. 3. *Case 2.* UTERINE CONTRACTIONS ON THE 2ND DAY OF THE CYCLE. The pressure inside the balloon is indicated on the left in mm. of mercury. The time interval in minutes is denoted below. Fig. 4. *Case 2.* UTERINE CONTRACTIONS ON THE 6TH DAY OF THE CYCLE.

DISCUSSION

In an earlier publication (4) the inadequacies of the current estrogen-withdrawal and progesterone-withdrawal theories were pointed out. The necessity of including some additional factor beside the two ovarian hormones in the mechanism of menstrual bleeding was emphasized. This extra but unknown element has been designated the 'bleeding factor' by several authors. At first, from the work of Hartman, Firor and Geiling (8), it seemed that the anterior lobe of the pituitary gland produced a special

hormone responsible for bleeding. This theory was challenged by Saiki (9). Finally Smith, Tyndale and Engle (10) demonstrated conclusively that the pituitary was not essential for menstrual bleeding. Zondek (11) also favored the concept of a bleeding factor but suggested that it might be a third ovarian hormone.

Menstruation, despite its dependence upon the ovarian secretions, is primarily a vascular phenomenon. The rôle of the uterine blood vessels in the mechanism of menstruation has been studied particularly by Bartelmez (12), Markee (13) and Daron (14). As a result of their pioneer work, it has been demonstrated that the

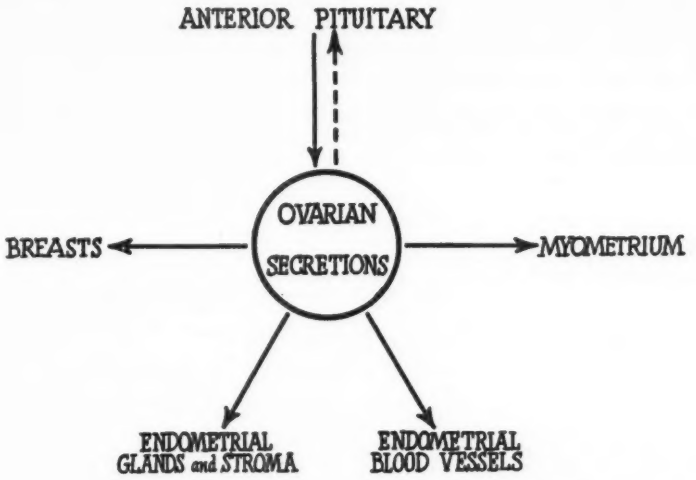


Fig. 5. The cyclic phenomena composing the menstrual cycle.

arterioles and capillaries of the endometrium undergo certain functional and morphological changes during the menstrual cycle. Brewer (15) recently stated that the skin capillaries participate in these changes but we have not been able to confirm his observations.

A clearer understanding of the events in the two cases described above may be had by referring to figure 5, which is a schematic representation of the various phenomena composing the menstrual cycle. The anterior pituitary controls and, in turn, is controlled by the ovarian secretions as are the breasts, myometrium, endometrium and the endometrial blood vessels. Yet these peripheral components do not exert direct control over each other. Normally certain temporal and quantitative relationships exist among the various components. Since the ovarian factor is constant in a given cycle, any derangement of the normal relations must result from variations in the reaction of the individual components.

In the cases described above the endometrial and vascular components were dissociated. In the second case the myometrium was also involved. Perhaps in some instances the breast and pituitary components might similarly become dissociated. This concept would explain the "unpredictability of the phenomena accompanying the menstrual cycle in normal women" (16). And the much-discussed bleeding factor is seen as the potentially variable reaction of the vascular component to the ovarian hormones. Thus the occurrence of menstrual bleeding depends not alone on the stimu-

lus supplied by the ovary but also on the reaction of the endometrial vessels to this stimulus. The particular type of endometrium present does not appear to influence this vascular reaction.

SUMMARY

Two cases in which a secretory endometrium was present on the sixth day of the menstrual cycle are reported. This finding is explained as a temporal dissociation of two of the several components of the menstrual cycle: the endometrium and the endometrial vessels. In one of the cases the myometrial component was also involved in the dissociation. A concept to account for the unpredictability of the phenomena accompanying the menstrual cycle in normal women is presented. The much-discussed 'bleeding factor' in menstruation is seen as the potentially variable reaction of the endometrial blood vessels.

We are indebted to Professor Benjamin P. Watson for constant encouragement and advice, and for placing the large material of the Vanderbilt Clinic at our disposal. We also appreciate the technical assistance rendered by Miss Jane Smelser.

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DETERMINATION OF pH OF VAGINAL SECRETION AS AN INDEX OF OVARIAN ACTIVITY IN HYPOOVARIAN STATES

JACOB S. BEILLY

From the Gynecological Endocrine Laboratory and Clinics, Department of Obstetrics and Gynecology, Beth-El Hospital

BROOKLYN, NEW YORK

Recently, the writer has pointed out a change of pH of vaginal secretion, from the usual acid toward an alkaline phase, in human castrates, menopause and hypoovarian groups, and its restoration, until the usual acid pH was attained, after the administration of adequate dosage of estrogens. Determination of the pH of the vaginal secretion was employed as a guide in treatment (1). The extent of the change of pH of the secretion was intimately associated with estrogen deficiency. Conversely, the degree of ovarian activity was ascertained. Thus, determination of the hydrogen ion concentration of the vaginal sample furnished an index of ovarian activity and served as an objective method of observation in the administration of estrogens.

The pH determinations were correlated with the cytology of vaginal secretion by the smear method of Papanicolaou and Shorr (2), and examinations of biopsy specimens of the vaginal wall (3). A better evaluation of the test of pH was obtained from these combined studies. The pH values of the secretion were found comparable with gradation of ovarian activity. Determination of the pH of the vaginal sample

TABLE 1. A COMPARISON OF pH AND CYTOLOGY OF VAGINAL SECRETION

pH range of vaginal secretion[1]	Classification of vaginal smears[2]	Histological criteria of vaginal smears					
		Clearness of smears or mucus	Blood cells or fibrin	Epithelial cells			
				Deep[3] cells	Vesicular nuclei	Pyknotic nuclei	Non-nucleated or cornified
5.4 to 8.0	I.	Clear	Leucocytes abundant	Abundant	Occasional	Occasional	Absent
5.0 to 8.0	II.	Clear	Leucocytes abundant	Many	Varying number	Varying number	Absent
4.6 to 7.0	IIa.	Clear	Leucocytes abundant	Varying number	Varying number	Predominant	Absent
4.4 to 6.4	IIb.	Clear	Leucocytes varying number	Occasional	Varying number	Predominant	Absent
4.0 to 5.4	III.	Clear	Leucocytes occasional	Absent	Occasional	Predominant	Occasional
4.0 to 4.4	IIIa.	Clear	Absent	Absent	Occasional	Predominant	Varying number
3.8 to 4.4	IIIb.	Clear	Absent	Absent	Absent	Occasional	Predominant

[1] See explanatory note on universal indicator in text.

[2] See photomicrographs for smear types.

[3] Characterized by Papanicolaou and Shorr (2). "Compact" cell of "negative smear" by Salmon and Frank (7).

TABLE 2. CASTRATE GROUP (SURGICAL, RADIUM AND X-RAY)

Case	Age	Significant data	Progynon-B dosage[1]	Changes in vaginal secretion			
				pH[2]		Cytology[3]	
				Before	After	Before	After
			R.U.				
1, J.S.	36	Post-castration, 3 yr.	40,000	6.0	4.0	I	III[b]
		Total hysterectomy with bi-lateral salpingo-oöphorectomy	30,000	6.0	4.0	II	III
2, F.H.	38	Castration syndrome, 6 months, following supra-cervical hysterectomy with bilateral salpingo-oöphorectomy	50,000	5.4	4.4	I	III
3, A.S.	50	Post-irradiation, 7 yr. intra-uterine radium	10,000	5.0	4.0	II	III
4, E.L.	43	Post-irradiation, 11 yr. with x-ray therapy	50,000	5.4	4.0	II	III[a]
5, P.P.	42	Post-irradiation, 4 mo. intra-uterine radium	none	6.0	—	I	—

[1] Progynon-B was generously supplied for this study through the kindness of Dr. W. Stoner of Schering Corporation, Bloomfield, New Jersey.
[2] See explanatory note on universal indicator in text. [3] See photomicrograph for smear types.

TABLE 3. MENOPAUSE

Case	Age	Significant data	Progynon-B dosage[1]	Changes in vaginal secretion			
				pH[2]		Cytology[3]	
				Before	After	Before	After
			R.U.				
6, I.F.	66	Menopause, complete, 23 yr. Hormone excretion,[4] FSH, present Ovarian H., absent	50,000	8.0	4.6	I	II
7, C.M.	48	Menopause, incomplete 3 yr. Severe menopause syndrome Hormone excretion,[4] FSH, negative Ovarian H.. absent B.M.R. +4	8,000	8.0	4.6	I	III[a]
8, L.W.	47	Menopause, incomplete, 1 yr. Severe menopause syndrome. Hormone excretion,[4] FSH, negative Ovarian H., absent B.M.R. −8	20,000	6.0	5.0	II[b]	III[a]
9, S.B.	46	Menopause, complete, 2 yr. Atrophic vaginitis. Hormone excretion,[4] FSH, present Ovarian H., absent	2,000	6.0	5.0	II[b]	III[a]
10, I.G.	55	Menopause, incomplete, 4 yr.	no estrogens thyroid med.	5.0	4.4	II	III
		Menopause syndrome. Hormone excretion,[4] Prolan, negative Ovarian H., 16 R.U./24. Total cholesterol, 384 mg.	only.	6.0	4.4	III[a]	III

[1,2,3] See table 2. [4] Darby and Kurzrok (10) method of hydrolysis.

TABLE 4. HYPOOVARIAN GROUP

Case	Age	Significant data	Progynon-B dosage[1]	Changes in vaginal secretion			
				pH[2]		Cytology[3]	
				Before	After	Before	After
			R.U.				
11, J.S.	18	General infantilism. Amenorrhea, primary Hormone excretion[4] FSH, positive Ovar. H., absent B.M.R. −3 Total cholesterol 319 mg. Tolerance test, hypoglycemic curve	54,000	8.0	—	I	—
				8.0	—	I	—
				8.0	4.4	I	III[a]
12, P.B.	27	Genital infantilism. Primary amenorrhea Hormone excretion[4] FSH, negative Ovar. H., 22 R.U./24 hr. B.M.R., −27	50,000	8.0	—	I	—
				8.0	—	I	—
				8.0	4.4	I	III
13, D.A.	23	Fröhlich syndrome Secondary amenorrhea, progressive from onset. Adiposity, body weight 235 lb. B.M.R. −12	none	7.0	—	II	—
14, B.G.	44	Fröhlich syndrome Amenorrhea, secondary progressive from onset.	30,000 thyroid	6.0	4.4	I	III
		Atrophic vaginitis Adiposity, body weight 243 lb.	44,000 thyroid	5.0	4.4	II	III
15, M.S.	31	Fröhlich syndrome Amenorrhea, secondary, progressive from onset. Adiposity, body weight 255 lb. B.M.R., +1. Hormone excretion[4] FSH, negative Ovar. H., no R.U. Total cholesterol, 237 mg. Hypoglycemic sugar curve. Glycosuria 1%	none	5.0	—	III	—
16, B.A.	29	Amenorrhea, secondary and overweight 35 lb. B.M.R., −15. X-ray of skull-hyperosteogeny site of coronal sutures. Slight degree of prognathism. Sella spherical.	none	5.4	—	II[b]	—
17, A.K.	25	Amenorrhea, secondary, progressive from onset of menses. Primary sterility. Serial endometrium, biopsies are in proliferative phase. Hormone excretion[4] FSH, negative Ovarian H., absent	none	6.0	—	II[b]	—
18, E.H.	29	Amenorrhea, secondary, progressive from onset of menses. Hypovarian syndrome. Atrophic endometrium Hormone excretion[4] FSH, present Ovar. H 4 R.U./24 hr. B.M.R., −12	none	5.0	—	III[a]	—
19, M.P.	27	Amenorrhea, secondary Obesity	none	5.0	—	III	—

[1,2,3] See Table 2. [4] Darby and Kurzrok (10) method of hydrolysis.

pointed to a better index of existing ovarian activity and was conducive in establishing a more flexible classification of vaginal smear types (table 1).

The test-method of determining the pH with universal indicator,[1] was found suitable for office and clinic observation. Several groups of cases are presented (tables 2, 3, 4) in which the determinations of the pH of the vaginal secretion was used as an index of ovarian activity and guide in treatment with estrogens.

DISCUSSION

Changes in hydrogen ion concentration of vaginal secretion seem to be intimately connected with ovarian activity, and the degree of ovarian activity bears a definite relationship to the consequent effect upon the acid-base balance of the secretion. This connection has been demonstrated by Miura (4), Lewis and Weinstein (5) and others, in man, and experimentally, in the rat (6). When the hormone mechanism which maintains normal vaginal effects, wanes or is interrupted, a diminishing acidity of the vaginal secretion follows.

The changes of pH seemed to follow closely the changes in cytology of the vaginal secretion before and after administration of estrogens. Similar vaginal effects were noted in the rat (7). However, the presence of an occasional 'deep' epithelial cell was encountered, frequently, in samples of vaginal contents of normal acidity. When numerous 'deep' epithelial cells were present, invariably, a diminishing acid-phase was found. The impression has been gained that the occurrence of an occasional 'deep' cell was not indicative of an atrophied epithelium present in the vagina. The reverse was true whenever there was a departure from the normal acid-base balance of the secretion.

Hydrogen ion concentration of the secretion furnished a true index of existing ovarian activity. Gradations of estrogen deficiency were recognized by the extent of the change of pH. Such pH values were comparable with changes in cytology of

[1] The universal indicator used in this work (Palo-Myers) passes through a long range of pH values (4.0 to 11.0 pH units) which can be recognized by distinctive, major color changes corresponding with larger variations of pH units, and intermediate, minor color changes with smaller pH units. When the color changes including the intermediate gradations are charted against more exact pH determinations, using indicators with buffered solutions (La Motte) a color chart can easily be constructed from which exact pH values can be read, directly, when using the universal indicator alone. Maximum error with universal indicator has been ± 0.4 pH units, when compared with colorimetric (La Motte) standards of the buffered and indicator solutions.

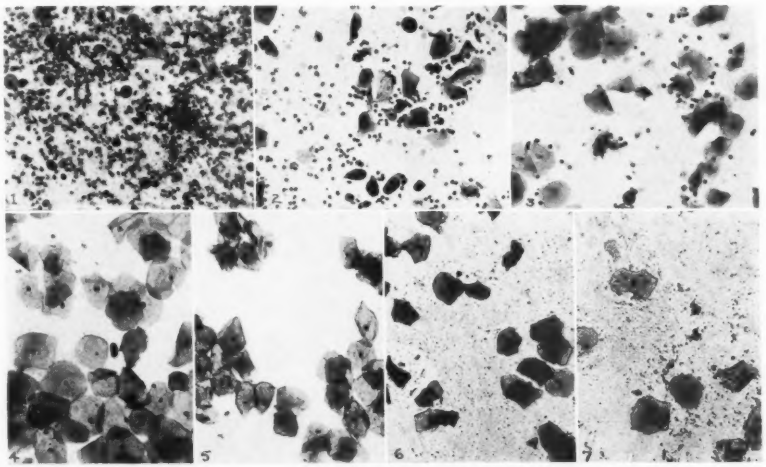
Universal indicator test solution is made upon the addition of several drops (3 to 5) of universal indicator in a test tube, previously dry-sterilized, and containing a small amount (¼ to ½ cc.) of normal saline solution. The test solution should be freshly prepared at the time, and should remain deeply green in color, and have a pH of 6.5 to 7.0 when no deterioration or contaminants, which effect a change of pH of the solution, are present. Caution as to the appearance of the indicator test solution, and checking with pH determinations should be heeded and observed at each session if correct readings of pH values are to be obtained.

Normal saline solution is used as diluent for universal indicator and in aspirating the vaginal secretion. It should be fresh and of pH 6.8 to 7.0.

Vaginal secretion can be aspirated, but when scant, the introduction of a small amount of normal saline solution (¼ to ½ cc.) in aspirator will help to obtain a uniform fluid sample of the vaginal contents for pH determination. The vaginal secretions appear to be well buffered and dilutions of 1 to 15 were found to retain the pH of the original sample. Within these limits, the original amount of vaginal secretion available is, therefore, of no moment.

With patient in lithotomy position, and with labia separated, before digital or instrumental examination, the vaginal aspirator, containing a small quantity (¼ to ½ cc.) of normal saline solution at its tip end is introduced at introitus and passed to the vault of the vagina, and its contents ejected. Allowing for spontaneous release of the bulb, it will aspirate the vaginal washings by slow suction. Upon removal of aspirator part of sample of contents is added to indicator test solution, and color change compared with color-chart of pH values, for direct reading. The remainder of sample can be tested against indicators with buffered solutions, for exact determinations. Vaginal smear slides should be prepared at the same time, and placed in alcohol-ether fixative solution for future staining and microscopic examination.

the secretion, principally in the character and numerical relationship of the epithelial cells, from the 'deep' or 'compact' cell to the non-nucleated or 'cornified' scales. Indices of vaginal smear types, suggested by Papanicolaou and Shorr (2, 8) and by Salmon and Frank (7) were found wanting for the identification of gradient estrogen deficiency when compared with determinations of pH of the vaginal secretion or biopsy specimens of the vaginal wall. These classifications, judged by the comparative data noted, are too rigid for a process essentially variable. We have, therefore, added



PHOTOMICROGRAPHS OF HUMAN VAGINAL SMEARS WITH RANGE OF pH OF VAGINAL SECRETIONS AS IN TABLE I. Fig. 1. Type I, pH 5.4 to 8.0; fig. 2. Type II, pH 5.0 to 8.0; fig. 3. Type II^a, pH 4.6 to 7.0; fig. 4. Type II^b, pH 4.4 to 6.4; fig. 5. Type III, pH 4.0 to 5.4; fig. 6. Type III^a, pH 4.0 to 4.4; fig. 7. Type III^b, pH 3.8 to 4.4. All Photomicrographs have been equally magnified ×103.

several sub-groups, retaining the major groups suggested by the above-noted authors (table 1).

Reding (9) observed, after treatment with estrogens, parenterally, no effect in normal women and an alteration in the pH of the blood of castrate, menopause and hypoovarian patients. We have found changes in the pH of the vaginal secretion, after treatment with estrogens, from the alkaline to the acid phase. Significant clinical improvement after estrogen administration (to be reported in detail, later) was invariably associated with large changes in pH units. These changes can be followed easily by the color changes produced with universal indicator, which have made the test applicable, clinically.

SUMMARY

Determination of pH of the vaginal secretion furnished an index of ovarian activity and served as an objective guide in the administration of estrogens in hypoovarian states. The simplicity of the test-method of determining the pH of the vaginal sample was found suitable as an office and clinic procedure.

A classification of smear types which was closely related to gradations of ovarian activity, and found comparable with hydrogen ion concentration of the vaginal secretion, is offered.

Several hypoovarian groups of cases are presented in which the test method of determining the hydrogen ion concentration of the vaginal secretion, with universal indicator, and vaginal smear observation were employed.

I am deeply indebted to Dr. Raphael Kurzrok of the Presbyterian Medical Center, New York City, and to Dr. Mendel Jacobi, director of laboratories, for their helpful suggestions. I highly appreciate the technical assistance rendered by Mr. Alvin Dubin.

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HORMONE EXCRETION AND BIOASSAY OF EXTIRPATED TUMOR IN TERATOMA OF THE TESTIS

JACOB S. BEILLY, IRVING KURLAND
AND MENDEL JACOBI

From the Department of Pathology and Gynecological Endocrine Laboratory of the Beth-El Hospital
BROOKLYN, NEW YORK

FROM A LABORATORY aspect of the case reported herein, one is impressed with the value of the biological method of testing in teratoma of the testes. In the light of the results obtained from serial examinations, it must be recognized as a valuable diagnostic procedure and as an assay of the effectiveness of treatment. This case is presented because it afforded such correlation of the bioassays and its clinical course, and assay of the extirpated tumor has suggested an interrelationship between hormone excretion and existing neoplasm.

L.S., a male, age 32, who was admitted to the hospital on 3/20/39, complained of persistent cough, joint pains and sore throat of 3 months duration. Hospitalization was advised for investigation, as there were remissions during that period of illness. Swelling of the right testicle had occurred 3 days prior to admission.

At the age of 3, he had had poliomyelitis which left deformities of the left lower extremity, and a marked kyphosis with unilateral deformity of the chest. Two years before the present admission, a hemorrhoidectomy had been performed.

Physical examination. The patient was a young male, with poor hemic component exhibiting deformities as described above. His temperature was 101°, pulse 108, respiration 24, B.P. 120/70. The throat was markedly congested. The chest was asymmetrical, anteriorly and posteriorly, with marked kyphosis of the left side. There was considerable dullness with diminished breath sounds in the left lower chest, posteriorly, at the point of greatest deformity, and hyper-resonance with considerable emphysematous breath sounds on the right side. Heart sounds were normal. There were no tender areas in the abdomen. Liver and spleen were not palpable. There was a large undescended and ectopic swollen testicle in the right inguinal canal. This could not be pushed down into the scrotum. Left testis was not palpable in the scrotum. Eye-ground examination results were completely negative. The x-ray examination of the chest revealed numerous well defined ovoid densities throughout the right pulmonic field. A metastatic malignancy with the primary focus in the right ectopic testicle was inferred, and a probable diagnosis of teratoma of testes was made.

Biological tests on urinary excretion confirmed the diagnosis of malignancy of the testes.

Laboratory Findings.

Blood count—4,300,000 R.B.C.	Blood chemistry—Glucose 92.0 mg.
11,600 W.B.C.	Urea N. 9.8 mg.
80% HB	Urinalysis—negative, sp. gr. 1018.
78% Poly.	Friedman test—positive pregnancy reaction.
22% Mono.	Aschheim-Zondek—positive pregnancy reaction.
	Aschheim-Zondek Quantitative test—FSH, 46,000 M. U. \ l.

TABLE 1

Date	X-ray treat. area	A–Z test			Remarks
		spec.	preg. test	prolan A M.U./l.	
3/20/39					Admitted to hospital.
					T. 101°, P. 108, R. 24, B.P. 120/70.
3/22/39					X-ray of chest: numerous well defined ovoid densities throughout rt. pulmonic field, suggesting metastatic malignancy.
					X-ray treatment begun.
3/23/39	rt. test	urine	pos.		Note: conc.-5x urine extract prepared according to Zondek method.
		conc.	pos.		
		quant		46,000	Preg.—pregnancy test.
					Friedman modification of A–Z strongly positive.
3/28/39	rt. test				x-ray treatment technic
	rt. lung				200 units, 200 Kilovolts
3/29/39	rt. test				4 Miliamperes
3/31/39	rt. lung				2 mm. copper filters
4/ 1/39	rt. test				2 mm. aluminum
4/ 3/49	rt. lung				40 cm. distance, 20 minutes
4/ 4/39	rt. test				10 cm.×15 cm. field, 2 ports
4/ 5/39	rt. lung				
4/ 7/39		urine	neg.		
		conc.	pos.	667	Operation, orchidectomy, right.
4/ 9/39		urine	neg.		
		conc.	pos.	13,200	
4/10/39		urine	neg.		Has lost weight and appears cachetic.
		conc.	pos.	667	Refused x-ray therapy.
4/11/39		urine	neg.		
		conc.	pos.	667	
4/12/39		urine	neg.		
		conc.	pos.	667	
4/15/39		urine	neg.		X-ray of chest: further extension in rt. pulmonic field, other small ovoid densities, not present before, have appeared, on left.
		conc.	pos.		
		quant		667	
4/17/39		urine	neg.		Discharged from hospital.
		conc.	neg.	667	Operative wound healed by primary union.
5/12/39		urine	neg.		Home, convalescence.
		conc.	neg.		Severe pain in chest requiring morphia to control symptoms; chills, nausea and vomiting, frequently.
		quant		13,200	
6/ 2/39					Readmitted to hospital.
					T. 99°, P. 92, R. 22, B.P. 124/92.
6/ 5/39					Marked loss in weight.
					X-ray of chest: numerous snow-ball-like metastatic infiltration through pulmonic field with increase in size as compared with previous exmination.
6/ 6/39	Chest				Postoperative x-ray treatment begun. Same technic as above except for time, 8½ minutes, at each session, alternately posterior and anterior.
6/ 8/39	Chest				
6/12/39	Chest				
6/14/39	Chest				
6/15/39		urine	pos.		
6/26/39		urine	pos.		Cachexia increased.
7/ 6/39		urine	pos.	33,300	
7/26/39					Died.

Progress. The patient was given a preoperative course of deep x-ray therapy from 3/23/39 to 4/7/39 (table 1). A dosage of 1000 r (roentgen units) to the testicle, and 800 r to the lung was administered before operation. A simple right-sided orchidectomy immediately followed. The left testis could be palpated in neither the scrotum

nor the inguinal canal. It was assumed to be located intraabdominally, but was not searched for.

Postoperative radiation therapy was refused by the patient, because of the marked reaction which had followed the previous course. He had lost weight and appeared cachetic. Subsequent x-ray examination, 10 days postoperatively, revealed further extension in the right lung. A small ovoid-shaped density, not in evidence on previous examination, was found in the left pulmonic field.

Two weeks after orchidectomy, the patient was discharged from the hospital with primary union, in care of his family physician. Postoperative radiation therapy was again refused because of increasing weakness, severe pains in the right lower chest, nausea and vomiting.

The patient was readmitted, 5 weeks later for a check-up and deep x-ray treatment (table 1). X-ray of the chest showed numerous snow-ball-like metastatic infiltrations throughout both pulmonic fields increasing in size, as compared with previous study. Examination of pelvis and skull revealed no evidence of further metastatic involvement.

Blood count—4,000,000 R.B.C.
11,200 W.B.C.
70% H.B.
70% Poly.
30% Lymph.

Blood chemistry—Glucose 76 mg.
Urea N 10 mg.

The patient continued to complain of pain in the chest, nausea and weakness. There was an icteric tinge to the sclera and his general condition was poor. A second course of deep x-ray treatment could not be carried out effectively because of the marked reaction and patient's refusal to receive this therapy. His condition became progressively worse; the cachexia increased, his pulse became rapid and weak. Quantitative estimations of the urine showed increasing quantities of follicle stimulating hormone. The patient became drowsy; chills and marked dyspnea developed and his pulse became feeble and rapid. The condition remained unchanged for 1 week; then the patient rapidly declined, and died.

Pathological examination of testicular tumor. The specimen consisted of testis and its covering with 12 cm. of vas and vessels attached. The entire mass weighed 100 gm. The testis measured $6\frac{1}{2} \times 5 \times 4$ cm. The surface was smooth and glistening but the entire testis, except for a small portion near the globus major, which portion still shows on section a grayish stringy appearance as seen in normal testis, was replaced by tissue which was irregularly soft and cystic, and firm and nodular. On section the soft cystic tissue was grayish and semi-gelatinous in appearance, contained small spaces in which were small amounts of thick mucus-like fluid but these spaces showed no gross lining membrane. The nodular areas were composed, in the more solid and firmer portions, of homogeneous yellowish tissue; while the softer nodules showed considerable central hemorrhagic infiltration and softening, and a few still softer nodular areas showed a caseous-like material composing the greater portion of the central half of the nodule. These nodules were fairly well demarcated from each other, but there was no gross suggestion of fibrous or other type of capsule, and between the nodules and cystic areas no gross testicular tissue could be seen. The remaining normal testicular tissue was infiltrated at its edges by the yellowish red tissue composing the major portion of the mass, and the epididymus was apparently completely replaced by firm yellowish tissue, while along the proximal two thirds of the vas, there was indurated yellowish tissue similar to that composing the mass, surrounding and apparently within the wall of the mass so that no gross lumen could be seen until the distal third of the vas was reached where the lumen and the wall

of the vas were grossly normal. In the areolar tissue surrounding the vas, no nodulations or gross neoplastic tissue could be seen.

Microscopical examination. Sections of the grossly definable testis showed shadowy outlines of tubules in which the basement membrane was thickened and hyalinized and no spermatic or Sertoli cells could be seen. These tubules were decidedly less in number than usual, were separated by wide areas in which fine fibrous reticulum was more prominent than usual and in which large numbers, both discreet and in patches, of hypertrophied Leydig cells were seen. The tumor described below invaded these sections. The epididymus showed a moderate intraductal fibrosis with the ductal epithelium somewhat hypertrophied and in places papillated slightly into the lumen of the ducts, but histologically intact.

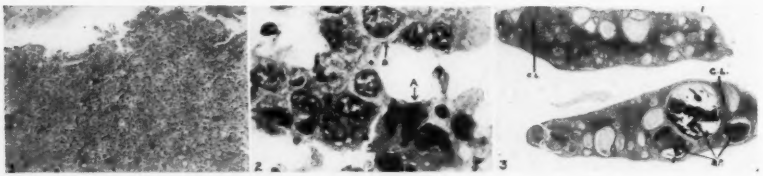

Fig. 1. Low power micro-photograph of testicular neoplasm showing syncytioma type of chorioepithelioma. The dark cells are trophoblasts, Fig. 2. High power micro-photograph of the tumor showing trophoblasts (A) and syncytial cells (B). Fig. 3. Rabbit ovary following injection of tumor-extract from tumor-bearing patient. Note comparatively few but markedly enlarged follicles. B.P., blood points ("Blutpunkte") or hemorrhages into follicles. C.L., corpora lutea.

Sections through the tumor showed a bizarre and variegated picture. The predominant picture was that of a reticulum-like arrangement of poorly defined, large flattened or cuboidal epithelial cells in which the cytoplasm was comparatively scant and vacuolated or poorly stained and in which the nuclei were enormous, moderately hyperchromatic and vesicular with distinct nucleolus and with frequent atypical mitotic figures. Scattered through such areas were compact areas in which the cells were roughly spindle-shaped, the cytoplasm fairly well stained and eosinophilic and fibrillar, and with the nuclei elongated, centrally located and moderately hyperchromatic; these areas strongly resembled smooth muscle. This was borne out by Mallory phosphotungstic acid stains. In one section could be seen a patch of compactly arranged, irregularly cuboidal cells in syncitial-like arrangement, these cells contained moderate amounts of deeply stained eosinophilic cytoplasm and a large, more or less centrally located markedly hyperchromatic nucleus (fig. 1). In these patches were also seen irregularly shaped giant cells occupied almost entirely by extremely large intensely stained and homogeneous nucleus of varied shape, mitotic figures being most frequent in both types of cells described in this patch, the larger cells in places suggesting a fusion of several cells. The entire patch was extremely similar to the type of tissue seen in chorioepithelioma of the syncitioma type, the large cells described resembling large hypertrophied and irregular trophoblasts (fig. 2). Numerous areas with considerable hemorrhage pepper all the sections. The pathological diagnosis was malignant teratoma of the testis containing chorioepitheliomatous elements, and showing post-radiation changes.

BIOASSAY

Excretion was determined by employing an adaptation of the Aschheim-Zondek test for pregnancy. Quantitative determinations were performed with dilutions of

urine, viz., 1 to 20; 1 to 50; 1 to 70; and 1 to 100; technic (number of injections, amounts and duration of test) was the same for all. Groups of 3 virgin mice, weighing 6.0 to 8.0 gm. were used in each assay; all ovaries were prepared for sections, reaction was ascertained on microscopic examination. The categoric reactions of Zondek (A.P.R. I, II and III) were used as a basis of end-results of titrations. Dilutions of urine were employed to minimize loss of material from injections. More uniform results were obtained with dilutions and when all ovaries were examined histologically. Concentrates were prepared according to the original method of Zondek. A comparison of the pregnancy test with untreated urine, and that obtained with concentrates, helped to evaluate fluctuations in hormone excretion during the course of illness. Computations were based upon equivalents of mouse units, per liter.

Hormone content of tumor was ascertained from a watery extract of a hemisection of extirpated tumor mass weighing 23.5 gm. The tissue was washed free from blood. It was finely minced, mashed and thoroughly admixed with 500 cc. of normal saline solution and then shaken, continuously, for 2 hours in a shaker machine. The supernatant fluid was filtered several times through paper filters until it was free from particles. Ether was used as preservative by over-laying a thin film on the surface of the fluid extract and kept in a refrigerator. Titrations were made using rabbits, rats and mice; in the latter 2, the technic employed was similar to that in hormone excretion. One gram of tumor mass assayed 142 mouse units of follicle stimulating hormone, 43 mouse units or 43 rabbit units of luteinizing hormone.

DISCUSSION

Zondek (1) was first to call attention to the abnormal hormone excretion of males in teratoma of the testes. An adaptation of the Aschheim-Zondek pregnancy test has become an indispensable diagnostic procedure in cases of suspected teratoma. Recession of hormone excretion is coincidental with recovery and reappearance of increasing quantities is indicative of recurrence or extension of the neoplastic disease. Quantitative determinations have since been employed to establish the diagnosis, and to evaluate the nature of the existing growth.

Metastatic infiltration of the pulmonic fields, in our case, suggested the nature of the disease, i.e., malignant neoplasm. However, its undoubted source was established by the biological test and finding of abnormal hormone excretion. Serial determinations were performed during the course of his illness (table 1) and hormone concentration followed closely the management of treatment. Thus, preceding irradiation treatment, the hormone excretion was 46,000 M.U. per liter, and the presence of luteinizing, as well as follicle stimulating effect, was demonstrable in undiluted urine with the Aschheim-Zondek and Friedman modification of the pregnancy test. After irradiation and orchidectomy, the hormone concentration dropped to 667 M.U. per liter of follicle stimulating factor, and although the Aschheim-Zondek test became negative, concentrates continued to produce both effects—follicle maturation and luteinization, as with untreated urine, before the combined treatment was carried out. Four weeks later, the hormone excretion had risen to 13,200 M.U. per liter; however, the pregnancy reaction had disappeared. The hormone concentration continued to rise, and 3 months later (beginning of patient's decline up to his death) excretion had risen to 33,300 M.U. per liter, and the luteinizing effect (pregnancy reaction) was again demonstrable in untreated urine.

According to Zondek, a positive Aschheim-Zondek test is due to the action of follicle stimulating hormone and the addition of luteinizing hormone. Demonstration of follicle stimulating hormone, alone, is not pathognomonic of pregnancy. From inception of pregnancy the earliest reaction begins with demonstration of follicle

stimulating hormone; subsequently, the luteinizing hormone effect appears. The reverse of this is true when pregnancy terminates; the luteinizing hormone effect is lost, first, and the follicle maturation effect remains last to disappear.

Histological examination of the mouse ovaries revealed a luteinizing effect (corpora lutea and corpora lutea atretica) present in assays of untreated urine, and later in concentrates only, up to discharge from the hospital, following x-ray treatment and operation. Four weeks following operation, luteinization was not demonstrable with untreated urine and concentrates, despite return of increased hormone elimination. Pregnancy reaction returned sometime later, 5 weeks before death.

Watery extract of tumor gave similar reaction as urine from the tumor-bearing patient. All the reactions were similar to those obtained with chorionic gonadotropic extracts. This was cause for speculation as to the nature of the neoplasm; whether or not the presence of either of the two gonadotropic hormones—follicle stimulating and luteinizing factors, bore any relation to the histopathology of the testicular growth. Hamburger (2) has pointed out marked differences of reaction in ovaries of the mouse and rat, from gonadotropins of chorionic and hypophyseal origin. The histological appearance of ovaries from assay of this tumor, was similar in its effects as those of chorionic gonadotropic fractions (fig. 3). Serial sectioning of the extirpated tumor revealed the presence of apparently incompletely developed fetal membranes.

Regression of hormone concentration and disappearance of the luteinizing effect, first, from untreated urine and later, from urine concentrates, followed the combined treatment of irradiation and orchidectomy. Increasing hormone excretion was concomitant with extension of the malignant process and increasing illness, and the luteinizing effect also returned, first with urine concentrates and subsequently with untreated urine.

This case poses the question as to the source of the two gonadotropic hormones as well as their effects upon the organism. We do not know whether they are part of the mechanism of attack on the part of the tumor, or of defense on the part of the organism.

SUMMARY

A case of malignant teratoma of testis containing chorio-epitheliomatous elements is reported, in which hormone excretion, before and after irradiation and orchidectomy, and hormone content of extirpated testicular tumor, were determined.

Hormone excretion was of value in the diagnosis of the character of testicular tumor and also furnished an index of the effectiveness of therapy.

It is suggested that luteinizing factor found preoperatively in urine and watery extract of tumor was related to the presence of incompletely developed fetal elements within the tumor.

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EFFECTS OF ANTERIOR PITUITARY EXTRACTS IN THE FASTED RAT

HELEN C. HARRISON[1] AND C. N. H. LONG
From the Laboratory of Physiological Chemistry, Yale University School of Medicine
NEW HAVEN, CONNECTICUT

AN INCREASED RETENTION of nitrogen in animals given extracts of the anterior pituitary containing the growth promoting fraction has been reported by several investigators. Teel and Cushing (1) observed that dogs on a constant diet showed a marked retention of nitrogen following the injection of an alkaline extract of anterior pituitary. Gaebler (2), as a result of carefully controlled balance studies, pointed out that the injection of an anterior pituitary extract into the normal dog kept on a high protein diet resulted in a decrease in urinary excretion of nitrogen which for periods of one or two days was as great as 50%. Lee and Schaffer (3), using the paired feeding technic, showed that rats treated with anterior pituitary extract gained more weight than their untreated controls. Analyses of the carcasses of the rats revealed a higher nitrogen content in the treated rats than in their littermate controls.

In addition to nitrogen balance studies the effect of anterior pituitary extracts on nitrogenous constituents of the blood has been investigated. Teel and Watkins (4) first showed that injection of a crude alkaline extract into dogs fasted 24 hours produced a decrease of 20 to 30% in the non-protein nitrogen of the blood. The fact that this decrease was not due to an increased excretion of nitrogen was shown by these authors. Teel and Cushing (1) and Gaebler (2) confirmed these findings. Mirsky and Swadesh (5) have reported a decreased production of nitrogenous metabolites in nephrectomized animals treated with alkaline anterior pituitary extract, and an increased protein catabolism, as indicated by blood N.P.N., in completely eviscerated animals.

In the experiments to be reported the effects of treatment with anterior pituitary extracts (APE) upon the endogenous protein catabolism of fasted intact rats and fasted adrenalectomized rats were studied. In this procedure, the total urinary excretion of nitrogen during the fasting period, and the blood N.P.N. at the end of the fast were determined. The excretion of ketone bodies in the urine and the effects of the extracts upon the blood sugar were also studied.

EXPERIMENTAL PROCEDURE

Male rats from a single colony, weighing between 125 and 175 gm., were used. Prior to the experimental period all the animals received the same stock diet, Purina Fox Chow. All animals were kept in individual metabolsim cages during the experiment. Twenty-four-hour urine samples were collected under toluene as accurately as possible, using a uniform technic of washing the cages. During the experiments the rats were fasted and allowed water ad libitum. The pituitary extract-treated animals received three 1 cc. sub-

[1] The data in this paper are taken in part from a dissertation presented by Helen C. Harrison in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Yale University, 1939.

cutaneous injections of crude APE daily, equivalent to approximately 420 mg. fresh gland. The 3 injections were spaced over a period of 12 hours. One group of intact rats was fasted 72 hours and received three 1 cc. injections of 0.9% sodium chloride solution daily on the second and third days; another group received injections of saline extracts of tissues prepared in the same manner as the saline extract of the pituitary. At the end of the experimental period, which was 48 or 72 hours, the rats were anesthetized with nembutal and blood was taken from the abdominal aorta for analysis.

Adrenalectomized rats were also studied in the same manner. They were adrenalectomized by the usual technic, using nembutal anesthesia. Subsequent to adrenalectomy they were offered the stock diet and a solution of sodium chloride and sodium bicarbonate (NaCl, 9.7%; $NaHCO_3$, 0.3%) ad libitum. The rats were weighed daily and only those whose preoperative weight was maintained or exceeded were used in the experiments. Five days postoperatively the experimental studies were begun. The rats were injected with a hypertonic solution of sodium chloride and sodium bicarbonate (NaCl, 1.4%; $NaHCO_3$, 0.6%) in a dosage of 5 cc. per 100 gm. body weight per day and were offered water ad libitum.

Blood glucose was determined by the Smogyi modification of the Shaffer, Hartman method (6) following precipitation of the protein with zinc sulphate (7); blood N.P.N. by the nesslerization after digestion with sulfuric acid and superoxol; urinary ketones by the Van Slyke method (8). All colorimetric determinations were made with a photoelectric colorimeter.

The pituitary extracts were prepared from beef anterior pituitary glands, which were frozen in carbon dioxide snow immediately upon removal from the animal at the slaughter house. The anterior lobe was carefully dissected from the remainder of the gland. The saline extract was prepared as follows: 14 gm. of anterior pituitary glands was ground in a mortar with a small amount of sand and extracted with 100 cc. of ice-cold 0.9% sodium chloride solution, centrifuged immediately and the supernatant fluid separated and stored in a frozen state. An alkaline extract of anterior pituitary glands was prepared according to the directions of Burn and Ling (9).

RESULTS

In table 1 the mean values for the daily nitrogen and ketone body excretion and the blood sugar and N.P.N. at the end of the experimental period of the treated

TABLE 1. EFFECTS OF SALINE AND ALKALINE APE ON NORMAL FASTING RATS

Group	No. of rats	Urine nitrogen mg./100 gm./day			Urine ketones mg./100 gm./day			Blood sugar mg./100 cc.	Blood N.P.N. mg./100 cc.
		1	2	3	1	2	3		
I. Saline controls	23	95±1.31	79± 2.63	84±4.70	0.4	0.7	1.2	84±1.88	41±1.94
II. Tissue extracts controls	6	94±4.51	92±12.05	115±5.70	—	0.3	—	102±4.82	43±2.38
III. Saline APE on 2nd and 3rd days	15	103±4.40	65± 2.46	58±2.26	0.5	17.7	12.6	57±2.30	29±1.01
IV. Saline APE on 1st and 2nd days	9	69±3.59	74± 2.11	—	0.6	22.3	—	55±4.30	34±1.06
V. Alkaline APE on 2nd and 3rd days	13	102±4.42	83±3.91	100±8.30	—	19.6	16.3	70±4.63	42±2.35

intact rats are given. Urinary excretions of nitrogen and ketones are expressed in terms of mg. per day per 100 gm. of body weight. The weight of the animals used in the calculations was that at the end of the first 24 hours of fasting.

Saline and tissue extract controls. Examination of the results shows that in the saline control group the mean nitrogen excretion on the second and third days of fasting is approximately 15% lower than that on the first day, although there was considerable variation among the animals. The fasted male rats excreted minimal

amounts of ketone bodies during the 3-day period. The nitrogen excretion of the animals given tissue extracts do not show the decrease on the second and third days found in the saline injected controls. Rather on the third day of fast there is a significant increase in nitrogen excretion. No ketonuria is produced by these extracts. The blood sugar at the end of the period of fast is higher in these animals than in the animals given saline. These effects are probably due to the injection of a foreign protein.

Effects of saline APE on normal fasted rats. The effects of saline APE given on the second and third days of fasting are shown in table 1. It is apparent that the extract produces a striking decrease in nitrogen excretion, the decrease being somewhat greater on the second day of administration. The mean excretions of nitrogen on the first and second days of extract administration are 37 and 44% lower, respectively, than the excretion on the preliminary day of fasting when no extract was given. When the urinary nitrogen values following administration of saline APE are compared with the nitrogen excretion of the saline-treated controls for the same days of fast there is found to be a decrease in nitrogen excretion of 18 and 31% for the second and third days of fast, respectively. If the nitrogen excretions of the animals receiving saline APE are compared with those of animals receiving saline extract of tissues, the decrease is much more pronounced. The blood N.P.N. is significantly reduced in the animals receiving saline APE as compared with the saline controls or tissue extract controls. There is also a marked ketonuria. Quite unexpectedly, the blood sugar values of the animals treated with saline APE are markedly lower than those of the controls.

The rats in Group IV (table 1) were fasted for 48 hours and treated with saline APE from the beginning of the fasting period. The dosage of extract used was the same as in the preceding experiments. In these experiments, the nitrogen excretion is again found to be decreased after saline APE injection as compared with fasted untreated animals. The decrease is significant only on the first day of treatment. The effect of the extract upon nitrogen metabolism is also shown by a diminished concentration of blood N.P.N. The concentration of blood sugar in this group is also low and of the same order of magnitude as that of Group III. The ketogenic effect of the extract is not apparent until the second day of treatment, indicating that a preliminary depletion of carbohydrate reserves is necessary before ketosis appears.

The effect of saline APE upon endogenous protein catabolism of fasting rats is strikingly shown in an experiment in which litter-mate rats at weaning were used. A litter of 6 female rats (Yale strain) which were all approximately the same body weight, was obtained. Two rats were killed at the beginning of the experiment, weighed, the carcasses dried at 105° to constant weight, the dried residue ground to a uniform powder and aliquot portions taken for fat analysis. The other 4 litter mates were kept in individual metabolism cages for 72 hours during which urine was collected under toluene for the entire period. Two of these rats were given two 0.5 cc. subcutaneous injections of saline APE daily during the fasts. The other 2 were given injections of 0.9% saline as controls. All 4 were killed at the same time, weighed, and the water and fat content of the dried carcasses determined. The total fat was determined by refluxing an aliquot of the dried powdered carcass with a hot alcohol ether solution for 1 hour; the solution was filtered and the alcohol ether evaporated off in front of an electric fan. The residue was extracted with petroleum ether and the petroleum ether-soluble material weighed after evaporation of the petroleum ether and drying at 105° for 10 minutes. The initial fat and water content of the fasted animals were assumed to be the same as that of the 2 litter mates which

were killed at the beginning of the experiment. The water and fat loss was then calculated by subtraction of the final water and fat content from an average of the initial values for these constituents. The protein loss was calculated by multiplying the total nitrogen excretion by the factor 6.25. The calories lost during fasting from protein and fat were also estimated.

The data are given in tables 2 and 3. A marked reduction in loss of protein of the animals treated with saline APE is apparent. Associated with this diminution of protein loss is a decreased water loss. This would indicate that loss of tissue water occurs simultaneously with loss of tissue protein. The difference in weight loss in the 2 groups is due chiefly to the difference in water loss since the disappearance of fat during fasting is approximately the same for the controls and the animals treated with saline APE. It is of interest that total fat breakdown is not increased during

TABLE 2. EFFECT OF SALINE APE ON PROTEIN AND FAT METABOLISM OF LITTER-MATE RATS

Group	Initial			Final			Urine	
	Wt. gm.	H_2O gm.	Fat gm.	Wt. gm.	H_2O gm.	Fat gm.	Nitrogen mg./period	Ketones mg./period
Controls	55	38.5	5.1	39	27.6	1.9	119.5	0.4
	55	38.5	5.1	39	27.9	2.0	133.5	0.7
Saline APE	55	38.5	5.1	44	32.6	1.9	94.0	13.7
	55	38.5	5.1	43	32.1	1.9	71.5	10.4

TABLE 3. CALCULATED LOSS OF TISSUE PROTEIN, FAT AND WATER

Group	Protein	Fat	Water	Calories (protein +fat)	Weight loss
	gm.	gm.	gm.		gm.
Controls	0.75	3.2	10.9	31.8	16
	0.83	3.1	10.6	31.2	16
Saline APE	0.59	3.2	5.9	31.0	11
	0.45	3.2	6.4	30.6	12

the brief period of treatment with saline APE even though a definite ketonuria is found. However, it is very likely that a shift of fat from depots to the liver did occur, in view of the findings of Best and Campbell (10).

The effects of treatment with alkaline APE on normal rats. The rats in Group V (table 1) were given the extract on the second and third days of fasting and the dose of the extract was the same as in the saline APE experiments. It is apparent that no decrease in nitrogen excretion is found with treatment with the Burn and Ling extract. In order to confirm the suspicion that the principle of the anterior pituitary responsible for the decreased nitrogen excretion was inactivated by treatment with alkali, a saline extract of the gland was prepared as already described and sufficient 1N sodium hydroxide solution added to make a final concentration of N/15 sodium hydroxide. This was allowed to stand overnight in the refrigerator, then neutralized to approximately PH 7.4 with 1N acetic acid. This alkalinized saline extract of anterior pituitary was given to 3 rats. In these animals no reduction in urinary excretion of nitrogen was found, although the original saline extract was effective in reducing nitrogen excretion. The concentrations of N.P.N. in the blood of animals treated with alkaline extracts of anterior pituitary are not reduced below the levels found in

fasting controls. The ketogenic activity of the extracts is as great as that of the saline APE. The blood sugar values of the rats treated with alkaline APE are significantly lower than those of the fasted controls, but are not reduced to the same extent as in the animals treated with saline APE.

Statistical treatment of data. In table 4 is demonstrated the statistical significance of the differences found between the variously treated groups, with respect to urinary nitrogen excretion and concentrations of blood sugar and N.P.N. In this table are given the differences between the means of each of the treated groups and the saline controls, plus or minus the standard error of the difference. In addition, for each of differences, the *P* value has been calculated by the method of Fisher (11) for small series. If the *P* values are 0.05 or less, the differences are considered to be significant. Examination of the results so presented conclusively demonstrates that the nitrogen excretion of fasted rats is considerably reduced following the administration of saline extracts of the anterior pituitary. Treatment with alkaline extracts

TABLE 4. STATISTICAL SIGNIFICANCE OF DIFFERENCES BETWEEN TREATED AND CONTROL GROUPS

Group	Urine nitrogen, days			Blood sugar	Blood N.P.N.
	1	2	3		
II. Tissue extract treated	− 1±4.70	+13±12.34	+31±7.40 $P<0.01$	+18±5.18 $P<0.02$	+ 2±3.07
III. Saline APE on 2nd and 3rd days of fasting	− 8±4.59	−14±3.56 $P<0.01$	−26±5.22 $P<0.01$	−27±2.97 $P<0.01$	−12±2.18 $P<0.01$
IV. Saline APE on 1st and 2nd days of fasting	−26±3.82 $P<0.01$	− 5± 3.37	—	−29±4.72 $P<0.01$	− 7±2.21 $P<0.02$
V. Alkaline APE on 2nd and 3rd days of fasting	− 1±6.3	− 4± 4.72	−16±9.54	−14±4.99 $P<0.02$	− 1±3.04

The values represent the differences between the indicated group and the saline treated controls.

of the anterior pituitary does not reduce urinary nitrogen excretion of fasted rats. That the effect of the saline extract is not a non-specific effect due to injection of foreign protein is shown by the fact that the saline extracts of other tissues do not decrease the nitrogen excretion but rather increase it. In all of the experiments in which reduction of nitrogen excretion is found there is also a significant drop in concentration of N.P.N. in the blood. Coincident with the reduction in nitrogen excretion in the saline APE experiments is found a markedly lowered concentration of blood sugar. The concentration of blood sugar is also found to be diminished in the rats treated with alkaline APE. However, the mean decrease in blood sugar produced by saline APE is significantly greater than that produced by the alkaline extracts. Both saline and alkaline extracts of the anterior pituitary induce a ketonuria in the fasted rats of approximately the same degree.

Adrenalectomized rats. In table 5 the results of the experiments on the adrenalectomized rats are given. The results of fasting alone deserve comment. In the fasted adrenalectomized rats the nitrogen excretion is significantly lower than in normal fasted rats and this decreased nitrogen excretion becomes progressively more marked on the second and third days of fasting. At the end of 48 or 72 hours of fasting, the blood sugars are found to be reduced considerably below the level found in the intact controls. In some cases marked hypoglycemia is found. The concentration of N.P.N. in the blood was not found to be elevated, with a few exceptions, indicating that the treatment with hypertonic solutions of sodium salts has been

adequate to maintain normal circulation and normal renal function, even though hypoglycemia develops.

Adrenalectomized rats treated with saline APE. The treatment of adrenalectomized rats with saline APE results in a further reduction of nitrogen excretion below the already low level found in the fasted adrenalectomized animals. The extract-treated rats also show a much more marked hypoglycemia than their controls. Many of these animals treated with saline APE show severe symptoms which can be explained by the hypoglycemia, i.e., low body temperature, lethargy and weakness. A number of the treated animals did not survive the 48-hour period of fasting and several of the animals seemed moribund at the time blood was taken for analysis. These animals showed the very low concentrations of blood sugar. Despite the marked hypoglycemia, these rats do not have elevated concentrations of N.P.N. in the blood. Actually, the blood N.P.N. concentrations are lower than in the fasted adrenalectomized rats not given saline APE. This is further evidence that the reduced nitrogen excretion shown by these animals is due to a diminished protein catabolism.

TABLE 5. EFFECTS OF SALINE APE ON ADRENALECTOMIZED RATS

Group	No. of rats	Urine nitrogen mg./100 gm./day			Urine ketones mg./100 gm./day			Blood sugar mg./100 cc.	Blood N.P.N. mg./100 cc.
		1	2	3	1	2	3		
I. Saline controls	23	95±1.31	79±2.63	84±4.70	0.4	0.7	1.2	84±1.88	41±1.94
VI. Adrenx. rats fasted 72 hr.	11	83±4.10	66±4.22	50±1.90	0.4	0.7	—	50±2.92	41±1.05
Difference (I–VI)		−12±4.31 P<0.02	−13±4.97 P<0.03	−34±5.07 P<0.01				−34±3.47 P<0.01	0
VII. Adrenx. rats fasted and given saline A.P.E. 1st and 2nd days of fasting	8	61±5.07	47±4.22	—	1.2	15.0	—	27±5.29	29±4.25
Difference (VI–VII)		−22±6.52 P=0.02	−19±5.96 P<0.02					−23±6.04 P<0.01	−12±4.38 P<0.05

In addition to producing a diminished nitrogen excretion and hypoglycemia, the injection of saline APE in the amounts indicated is found to produce a definite and marked ketonuria.

DISCUSSION

In the experiments reported here, administration of a crude saline APE has been shown to produce a decrease in endogenous protein catabolism in the normal fasted rat, as well as in the adrenalectomized rat in whom nitrogen excretion is already low. In previous experiments on the effects of APE in fed dogs (2) and rats (3) the nitrogen retention demonstrated was thought to be correlated with the growth promoting activity of the extracts used. The simplest explanation for the results obtained in the present experiments in the fasted rat would therefore be that the growth promoting factor of the anterior pituitary inhibits protein catabolism even in fasting animals, resulting in a diminished oxidation of tissue protein. It is of interest that this effect of APE upon nitrogen excretion is found in the adrenalectomized animal, since adrenalectomy has been shown to abolish the diabetogenic effects of APE.

In contrast to the nitrogen retention effect, experimental evidence for a stimulation of protein break-down by crude APE has also been offered by several investigators. Houssay (12) has shown that crude APE increases protein catabolism in fasting hypophysectomized-depancreatized dogs; in other words, an effect opposite

to that found in intact fasted rats. Mirsky and Swadesh (5) in a preliminary report have indicated that in dogs inhibition of protein catabolism by APE does not occur in depancreatized animals, whereas this effect can be demonstrated in animals with intact pancreas. No evidence is at hand to show what effect on nitrogen excretion would be exerted by crude APE in the fasting depancreatized rat. Mirsky and Swadesh have also reported that in the totally eviscerated dog, injection of APE results in an increased blood N.P.N., which is interpreted as evidence of another action of the pituitary on protein metabolism, i.e., a direct stimulation of protein metabolism in the muscle. If there is such a dual effect, it is obvious that in the fasting intact rat, as well as in the fasting adrenalectomized rat, the inhibitory effect on protein catabolism is the predominant one brought about by treatment with saline APE.

It is of interest that APE may be prepared which possess ketogenic activity but no longer show any effect upon the nitrogen metabolism of the fasted rat. In the present experiments alkaline extracts of the anterior pituitary prepared according to the directions of Burn and Ling have not been found to exert an inhibitory effect upon protein catabolism in the fasting rat.

The finding of hypoglycemia in rats treated with APE was surprising in view of the fact that in recent years pituitary extracts have been found to produce persistent hyperglycemia in dogs (13–17). Houssay (18) found that hyperglycemia and glycosuria could be produced by these extracts only in fed animals. Young (19) has reported that the rat is practically insensitive to this so-called 'diabetogenic' action of the APE.

At present the important rôle of the anterior pituitary in carbohydrate metabolism is generally accepted, but the mechanism of its action is still unexplained. The original observation of Houssay that hypophysectomy ameliorates the diabetes of depancreatized dogs and that hypophysectomized animals are markedly sensitive to insulin led to the view that the anterior pituitary stimulates gluconeogenesis. The diminished glycosuria following hypophysectomy in depancreatized dogs, the spontaneous hypoglycemia in fasted hypophysectomized animals, and the insulin sensitivity of these animals were explained on the basis of diminished production of glucose from non-carbohydrate sources. The work of Long and Lukens (20) has indicated that atrophy of the adrenal cortex, following hypophysectomy, may be an important factor in these disturbances of carbohydrate metabolism. However, considerable evidence has been presented which indicates that oxidation of carbohydrate is increased in hypophysectomized animals and that AP preparations may diminish such oxidation. The evidence for such a view has been well summarized by Russell (21).

The hypoglycemia found in the present experiments in fasted rats treated with large doses of saline APE does not fit in with any of the theories discussed above. The finding of a coincident diminution of nitrogen excretion and decreased concentration of blood sugar in fasting rats treated with saline APE suggests that both effects may be explained by an inhibition of protein catabolism and decreased gluconeogenesis from protein.

Soskin, Mirsky, Zimmerman and Crohn (22) have suggested that in the normal animal glucose may be derived from both protein and fat, whereas in the hypophysectomized animal, gluconeogenesis from fat does not occur. Their contention that gluconeogenesis from fat may occur in the normal animal is not supported by any conclusive evidence. In the present experiments fasting normal animals given APE developed hypoglycemia even though fat metabolism in the liver was stimulated as indicated by the production of ketosis. The coexistence of ketosis and hypo-

glycemia would indicate that under these conditions blood sugar cannot be maintained by utilization of body fat.

Adrenalectomized rats, maintained with sodium salts, show a marked reduction in urinary excretion of nitrogen during fasting as compared with intact rats. A decreased nitrogen excretion in the fasting adrenalectomized rat has been previously reported by G. T. Evans (23), and the order of magnitude of the decrease agrees well with the present results. These findings lend support to the hypothesis that the hypoglycemia found in fasted adrenalectomized rats is due to a diminished gluconeogenesis from protein.

SUMMARY

Administration of saline extracts of the anterior pituitary to fasting rats results in a reduction in urinary excretion of nitrogen and a decreased concentration of blood N.P.N. There is also found a marked ketonuria and a considerable decrease in the concentration of blood sugar.

Alkaline extracts of the anterior pituitary, prepared according to the directions of Burn and Ling, do not produce any significant effect upon the nitrogen metabolism of fasted rats but do exert a marked ketogenic activity.

Adrenalectomized rats, fasted and given large amounts of sodium chloride and sodium bicarbonate by intraperitoneal injection, develop hypoglycemia. These animals also show a considerable reduction in nitrogen excretion as compared with fasted intact rats. The injection of saline extracts of the anterior pituitary into adrenalectomized rats produces a further decrease in nitrogen excretion and a more marked hypoglycemia. These animals also show a definite ketonuria.

The association of a decreased nitrogen excretion and hypoglycemia suggests that the reduction in blood sugar may be the result of diminished gluconeogenesis from protein due to an inhibition of protein catabolism. The changes in blood sugar found in fasted adrenalectomized rats may also be explained on the basis of inadequate gluconeogenesis.

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EXPERIMENTAL STUDIES OF THE ANTERIOR PITUITARY

V. FUNCTIONAL ACTIVITY OF ANTERIOR PITUITARY GRAFTS IN THE ADULT MALE GUINEA PIG

M. SCHWEIZER, H. A. CHARIPPER AND W. KLEINBERG[1]

From the Department of Biology, Washington Square College, New York University

NEW YORK CITY

IT IS GENERALLY AGREED that the female pituitary secretes two gonadotropic substances, one a follicle stimulating and the other a luteinizing hormone; but it is by no means accepted that both of these hormones are necessary for the maintenance of the male reproductive system. It is generally believed that the follicle stimulating hormone maintains the germinal epithelium of the testis and the luteinizing hormone is necessary for the maintenance of the interstitial tissue (1). That this explanation is not entirely adequate is indicated by a later report of Greep and Fevold (2) that the luteinizing hormone alone may stimulate both the interstitial and the reproductive elements of the testis. Cutuly, McCullagh and Cutuly (3) obtained results with parabiotic unions between hypophysectomized male rats and castrated, cryptorchid or normal partners which were believed to be interpretable on the basis that one hormone could account for maintenance of the testis as both an androgenic and a reproductive organ.

Observations on the activity of intra-ocular transplants (homoplastic) of the anterior pituitary in the hypophysectomized male guinea pig form the basis of the present communication. An earlier investigation (4) of the behavior of such implants in the hypophysectomized female showed that the grafts apparently secreted only follicle stimulating hormone. The intra-ocular transplant, therefore, seemed to offer a convenient means of observing the effect of endogenous FSH on the testis.

EXPERIMENTAL PROCEDURE

The method used was essentially that previously described (4, 5). Adult male guinea pigs (350 to 500 gm.) were hypophysectomized by the parapharyngeal approach. The anterior pituitary of a freshly killed male or female was immediately implanted into the anterior eye chamber. Animals were killed at varying intervals from 59 to 156 days after operation. At the time of killing the sella was exposed and any fragments were carefully collected and later examined in serial section.

The following criteria were used to determine the functional state of the implants.

(*a*) *Gross appearance.* The animals were weighed and the general appearance of the genitalia examined at regular intervals. The size of the horny styles served as a useful index of gonadotropic activity, as these practically disappear after complete hypophysectomy. The size and state of distension of the seminal vesicles was observed at autopsy.

(*b*) *Organ weights.* The testes and adrenals were weighed before fixation.

[1] Technical assistant

(*c*) *Electrical ejaculation test.* In cases in which electrical ejaculation tests were performed, the amount and consistency of the ejaculate was observed. The presence of motile sperm in the ejaculates was determined by microscopic examination of the coagulum mixed with saline. It was not possible to use this test routinely, since the electrical shock in some instances proved fatal to the animals.

(*d*) *Histological examination.* At autopsy the testes, seminal vesicles epididymides, adrenals and eyes were prepared for histological study.

Controls were of two types: six were hypophysectomized unimplanted animals killed at intervals from 59 to 139 days after operation; four were hypophysectomized, implanted animals in which the implants had failed to take. So far as the effects on the gonads and on the adrenals are concerned these two groups may be considered as one.

RESULTS

A summary of the experiments is given in table 1. Animals designated *MM* had implants of pituitary tissue from a male, those designated *MF* had grafts from a female donor.

The experimental animals have been divided into two groups: group *A*, completely hypophysectomized (representing those cases in which no trace of anterior lobe tissue was found in the sella); group *B*, incompletely hypophysectomized (including those cases in which any fragments of anterior lobe were present). The size of these fragments was estimated; the largest, (*MM6*), was well below 1/10th of a normal gland; the smallest, (*MM2*), was merely a patch of anterior lobe cells imbedded in a bit of pars intermedia. The size of the fragments was well below the limits generally considered necessary to maintain an animal (6, 7). The fact that the adrenal weights of group *B* did not differ from those in the control series lends force to the suggestion that these fragments were too small of themselves to have prevented the usual hypophysectomy atrophy.

All of the hypophysectomized guinea pigs with successful ocular transplants possessed testes in which the reproductive elements and interstitial cells were stimulated.

That spermatogenesis was well maintained was evidenced by the fact that *a*), In the 6 experimental animals (*MM2*, *MM3*, *MM4*, *MM6*, *MF3*, *MF5*) in which electrical ejaculation tests were performed motile sperm were present in the ejaculate; *b*), The tubules of the testes showed all stages of spermatogenesis and the epididymides were well filled with sperm (fig. 3, 7, 11, 12).

That androgenic activity was maintained was indicated by the following: *a*), the firm, well descended testes, the horny styles of normal size (table 1) and the large seminal vesicles distended with viscous fluid; *b*), the 6 experimental animals tested by the electrical ejaculation test gave copious viscous ejaculates which soon coagulated; *c*), the epithelium of the seminal vesicles was well maintained and as high as in the normal animal. The interstitial tissue of the experimental animal was not as abundant as in the normal but the cells of this tissue were normal and healthy in appearance (fig. 3, 7, 11, 12).

There was no detectable difference in the maintenance of either spermatogenic or androgenic activity whether the implanted pituitary was from a male or from a female donor.

In contrast to the clear-cut effect observed in the gonads no definite evidence of any stimulation was noticed in the sections of the adrenals.

Although the number of cases is small, a comparison of the testis figures for group *A* and *B* (table 1) suggests that the difference in weights might be significant. Pitui-

TABLE I

Group	No.	Days Hypophysectomized	Body wt. at death gm.	% Gain	Testis wt. gm./100 gm. body wt.	Adrenal wt. gm./100 gm. body wt.	Horny styles, mm.
Controls							
Hypophysectomized	134	59	403	1	.07	.035	
	135	59	391	−7	.30	.029	<1.0
	136	59	416	−9	.09	.031	
	137	59	433	3	.38	.028	
	128	120	392	7	.23		
	133	139	384	10	.35	.027	<1.0
Hypophysectomized	M1C	76	615	90	.05	.031	<1.0
	M2C	77	755	40	.06	.041	1.0
	MM1	68	680	35	.20	.036	1.0
Implanted Non-takes	MM7	94	780	70	.04	.023	1.0
Average ± A.D.[1]					.18 ± .12	.031 ± .004	
Experimentals Group A							
Completely Hypophysectomized	MM3	78	795	20	.37	.032	2.0
	MM4	130	605	30	.44	.021	4.5
	MM5	132	525	38	.30	.032	2.5
	MF1	114	520	57	.40	.044	2.5
Average ± A.D.					.38 ± .04	.032 ± .006	
Group B							
Incompletely Hypophysectomized	MM2	77	530	15	.57	.039	
	MM6	105	550	37	.69	.027	4.0
	MF2	104	490	25	.54	.030	4.0
	MF3	113	590	75	.54	.046	2.5
	MF4	104	620	70	.57	.038	4.0
	MF5	156	550	63	.57	.032	3.5
Average ± A.D.					.58 ± .04	.035 ± .006	
Normals			455		.48	.057	2.0
			490		.50	.062	
			620		.76	.085	4.0
			810		.46	.067	5.0
			675		.63	.053	5.0
			635		.53	.057	4.0
			575		.69	.077	3.5
			585		.53	.038	4.0
			615		.60	.108	4.0
			500		.64	.089	4.0
			520		.44	.041	4.0
			550		.42	.057	3.0
			560		.46	.051	3.5
			590		.49	.048	4.0
			520		.45	.037	4.0
			580		.46	.042	3.5
			575		.62	.052	4.0
Average ± A.D.					.54 ± .08	.060 ± .015	

[1] Average deviation.

tary fragments in the sella, although inadequate for adrenal maintenance, appear to have been effective in augmenting to some extent the gonadotropic effect of the implants.

A puzzling circumstance was the increase in weight (due to proportional growth and not to obesity) in 4 of the control animals (*M1C*, *M2C*, *MM1*, *MM7*). This striking increase occurred only in those guinea pigs of the control series which had been implanted and in which the implants had failed to take. It might be supposed that the increase occurred in the initial stages of the experiment before the implants had regressed, but actually, the weights of these animals showed a steady increment until the time of death, although in all other respects they displayed the typical effects of hypophysectomy.

DISCUSSION

Anterior pituitary tissue homoplastically transplanted into the anterior eye chamber of the hypophysectomized female guinea pig apparently secretes only follicle stimulating hormone (4). The ovarian follicles are well maintained and liberate sufficient estrogenic hormone to produce enlarged uteri and prolonged estrous openings of the vagina. There is no evidence, however, that such implants secrete luteinizing hormone since the follicles fail to reach maximum size and neither ovulation nor formation of luteal tissue occurs.

In extending our experiments to the hypophysectomized male we had anticipated again obtaining a purely FSH effect from the transplants. However, it is apparent from the results obtained that the grafts maintained the androgenic as well as the spermatogenic activity of the testis. This cannot be attributed to a sex difference in the implants, as the effects of pituitary from male and female donors were indistinguishable.

These results are essentially in agreement with those of Cutuly (8) who has made auto-transplants of anterior pituitary in the anterior eye chamber of hypophysectomized male rats. About 150 days after operation these animals showed moderate to good stimulation of the seminiferous tubules, but poor stimulation of the interstitial cells of the testes. There was no observable stimulation of the adrenals or the thyroid and no general body growth.

There is also marked similarity between these results and those of Pfeiffer (9) who has reported that the masculinized pituitaries of female rats stimulate only follicular development in the ovary but maintain both the spermatogenic and the interstitial tissue of the testis. Pfeiffer interprets these results as indicating that FSH alone is able to maintain the male gonad. Greep (10) questions this interpretation and has shown that extracts of "pure" FSH will maintain only the germinal elements of the rat testis, when it is given in minimal amounts, whereas when much greater doses are administered the interstitial tissue as well as the spermatogenic tissue of the testis is stimulated. Greep believes that this effect is due to contaminating traces of LH in the extract and that the male gonad is an unusually sensitive test for even very small amounts of this hormone.

There is as yet no conclusive means by which to evaluate these results. Several possible interpretations suggest themselves; one may assume, as Pfeiffer suggests, that the male pituitary while possessing small amounts of LH does not normally secrete this hormone and that the male gonad can be maintained by FSH alone. Or one may be equally justified in adopting a unitarian point of view, explaining the difference in response between ovary and testis, on the one hand, and germinal and interstitial tissue on the other, upon a purely quantitative basis, assuming a tissue threshold variation. Finally one may accept Greep's interpretation, which may be said to agree nicely with the present results. The effects of the implants in the hypophysectomized female guinea pig indicated that FSH was secreted in adequate amounts but that if any LH was liberated it was in quantities too minute to produce

any detectable effect on the ovary. The presence of less than normal amounts of interstitial tissue in the testis of the hypophysectomized, implanted male guinea pig indicates that the hormone stimulating this tissue was not being secreted at normal levels. This suggests that though the implants secrete ample amounts of FSH,

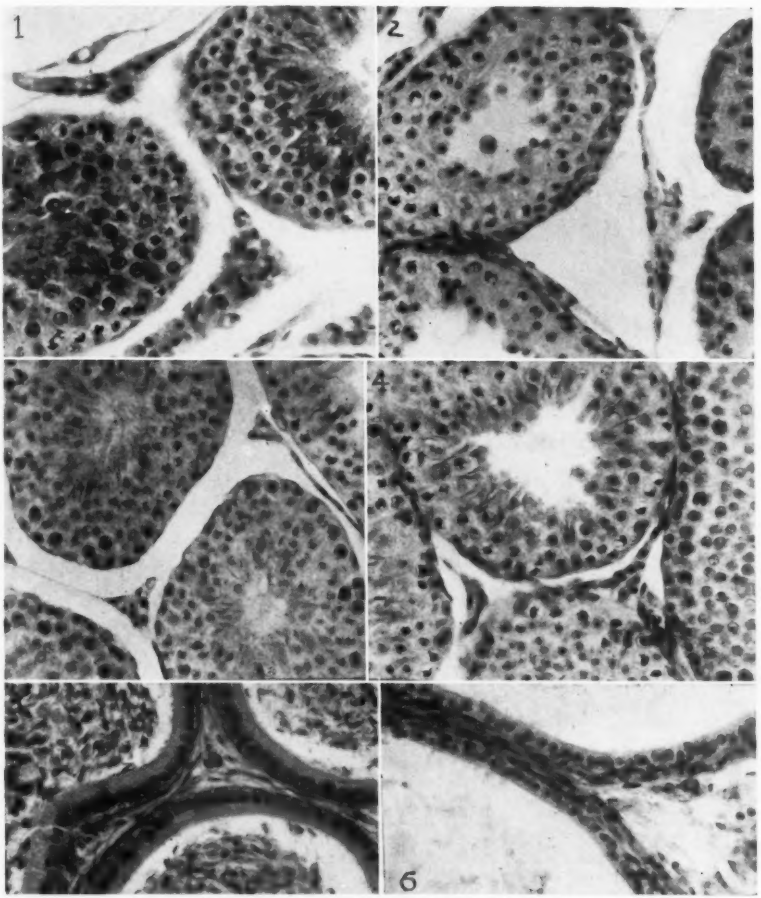

Fig. 1. Testis. Normal adult guinea pig. Fig. 2. Testis. Hypophysectomized control, *No.* 136, 59 days after hypophysectomy. Fig. 3. Testis. Hypophysectomized and implanted with male pituitary *No. MM4*, 130 days after hypophysectomy. Fig. 4. Testis. Hypophysectomized and implanted with female pituitary *No. MF1*, 114 days after hypophysectomy. Fig. 5. Epididymis. Normal. Fig. 6. Epididymis. Control, *No.* 136. (All figures are photomicrographs of tissues fixed in Bouins and stained in haemotoxylin-eosin. ×260.)

they pour out quantities of LH below the threshold necessary to effect luteinization or ovulation in the ovary but sufficient to stimulate to some extent the more sensitive interstitial tissue of the male gonad.

Of interest in this connection is the recent work of Eversole, Edelmann and Gaunt (11) on the function of grafts of adrenal cortical tissue in adrenalectomized

rats placed under conditions of stress. The grafts secreted sufficient hormone to keep the adrenalectomized animals alive but were not able quickly to put out enough hormone to assure a normal recovery from the stress of water intoxication. It is tempting to suggest in the present work that the pituitary grafts, in a similar way, were able

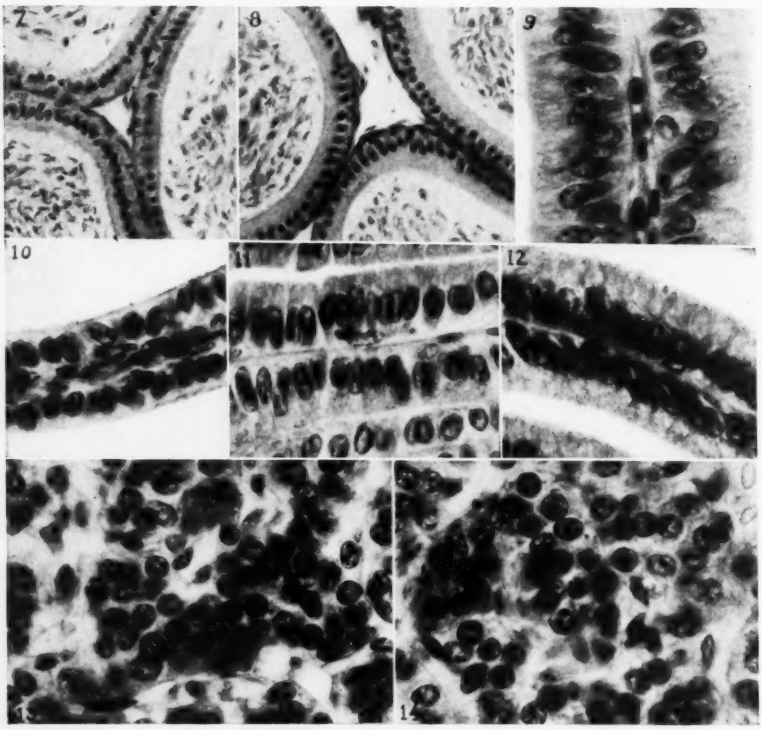

Fig. 7. Epididymis. Hypophysectomized and implanted, *No. MM4*. ×225. Fig. 8. Epididymis. Hypophysectomized and implanted, *No. MF1*. ×225. Fig. 9. Seminal vesicle epithelium. Normal ×580. Fig. 10. Seminal vesicle epithelium. Control. *No. 136* ×580. Fig. 11. Seminal vesicle epithelium. Hypophysectomized and implanted *No. MM4*. ×580. Fig. 12. Seminal vesicle epithelium. Hypophysectomized and implanted *No. MF1*. ×580. Fig. 13. Anterior pituitary from intraocular implant. *No. MM4*. ×580. Fig. 14. Anterior pituitary from intraocular implant. *No. MF1*. ×580.

to secrete steadily small amounts of LH but were not able to mobilize periodically the larger quantities of this hormone probably necessary for the cyclic responses of the ovary.

SUMMARY

Hypophysectomized male guinea pigs with intra-ocular transplants (homoplastic) of anterior pituitary tissue had well maintained reproductive systems. The occurrence of all stages of spermatogenesis in the testes, epididymides well filled with sperm and the presence of motile sperm in the ejaculates indicated maintenance of the germinal epithelium. Although the interstitial tissue of the testis was less abundant than that in the normal guinea pig, the individual cells of this tissue were normal

in appearance. There was definite evidence of androgenic activity in the large size and distended state of the seminal vesicles, the size of horny styles and the positive results of the electrical ejaculation tests. The germinal and the interstitial tissue of the testis was maintained to the same extent whether the implant tissue was obtained from a male or from a female donor. The bearing of these observations upon current interpretations of certain phases of the gonad-pituitary relation are discussed.

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PHOTOELECTRIC PLETHYSMOGRAPHY OF THE FEMALE BREAST

SAM. GORDON BERKOW AND MURRAY B. JACOBSON
PERTH AMBOY, NEW JERSEY

VARIATIONS in the blood content of the female breast are of special interest because the vascularity of this part may be influenced physiologically (by puberty, the menstrual or ovulatory cycle, pregnancy, lactation, menopause) either by or with relation to the sex hormones. Breast pathology (engorgement, mastitis, tumors) also produces distinct circulatory changes.

Several investigators have reported the use of photoelectric cells for the detection of changes in the blood content of tissues. Hertzman (1) reported "the development of

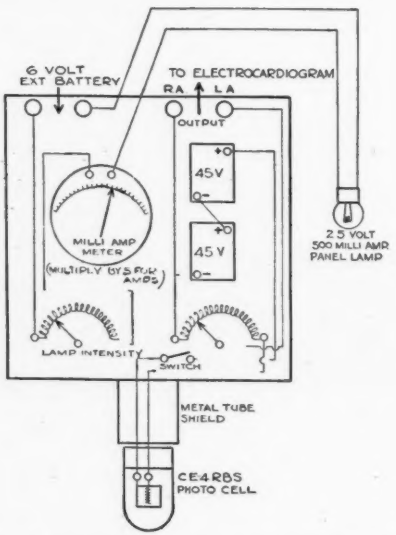

Fig. 1. PHOTOELECTRIC PLETHYSMOGRAPH, DIAGRAMMATIC.

photoelectric plethysmographs which lend themselves to clinical use in dynamic analysis of the peripheral circulation and which may be 'plugged in' in place of the patient's leads on the electrocardiograph." The photoelectric plethysmograph used by us (fig. 1, 2) was adapted from various arrangements described by Hertzman (2).

The breast is illuminated by a 2.5 volt 500 milliampere (radio panel) bulb, powered by 4 dry cells (6 volts). This is coupled with a rheostat and a milliammeter, through which the current generated in the lamp can be kept constant. (In this series it was kept at 750 milliamperes.) The lamp is shielded by a firm, slightly flexible rubber cap. A pivot just above the lamp housing permits angulation, so that the rubber cap can be approximated to various breast curvatures. This is attached to a spring-rod, to

measure pressure, and a movable extension to measure distance (ours was made from the mechanical stage of a microscope). Light transmitted through the breast is received by a photoelectric cell (Centron CE-RBS). Amplification is regulated by a rheostat connected with two 45-volt batteries. Variations in the photoelectric current, thus amplified to the desired degree, are recorded by an electrocardiograph.

Preliminary studies to determine the effects of pressure and distance showed that

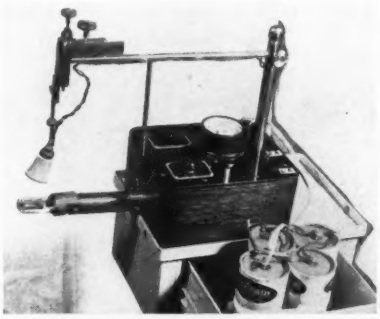

Fig. 2. PHOTOELECTRIC PLETHYSMOGRAPH.

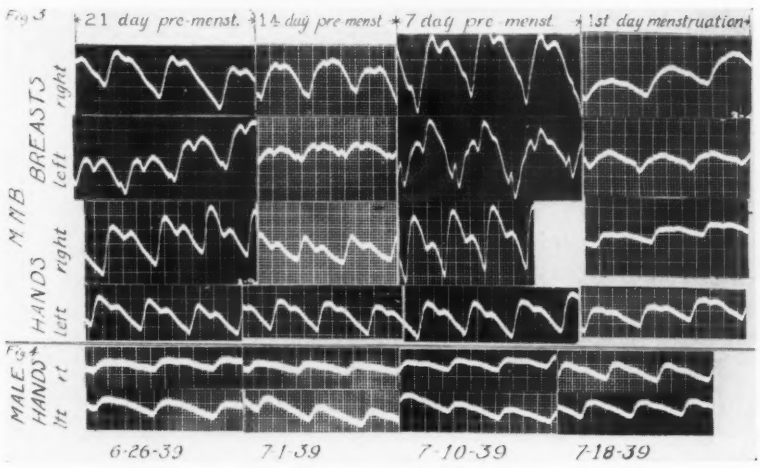

Fig. 3 and Fig. 4. PLETHYSMOGRAPH CURVES.

these influenced the amplitude of the curve without essentially altering its form. These findings, together with transillumination of diverse breast areas, resulted in the present technic. The photoelectric cell is placed under the breast. The light is screwed down just above the areola, overlying the photoelectric cell, with just sufficient pressure to make firm application.

To determine the normal variation of breast circulation, two subjects were studied; one with a regular 28-day cycle (*V. B.*), the other having an irregular 30 to 35-day cycle (*M. M. B.*). Curves were obtained from each breast for 2 months (January to March, 1939), at intervals of 1 to 3 days. Thereafter, on the advice of Dr. Robert T.

Frank, curves were obtained in the same subjects by transillumination of the web between the thumb and the index finger of either hand in conjunction (not simultaneously) with the breast curves. Figure 3 shows the curves from the right and left breasts of *M. M. B.* during menstruation, and intermenstrually at intervals of 1, 2 and 3 weeks, and the hand circulation at these times. In this subject each breast showed its own distinctive curve which was superimposed upon other changes recorded

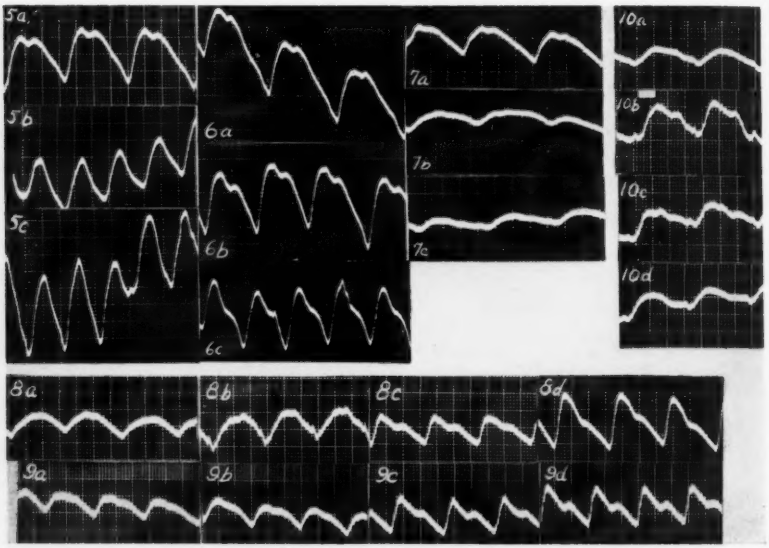

Fig. 5*a*, 5*b* and 5c. Effects of amyl nitrite: 5*a*, before inhalation; 5*b*, 20 seconds after inhalation; 5c, 30 seconds after inhalation.

Fig. 6*a*, 6*b* and 6c. Effects of smoking: 6*a*, before smoking; 6*b*, smoking for 90 seconds; 6c, smoking for 3 minutes.

Fig. 7*a*, 7*b* and 7c. Effects of constriction of breast by elastic band: 7*a*, before constriction; 7*b*, constriction for 1 minute; 7c, constriction for 3 minutes.

Fig. 8*a*, 8*b*, 8c and 8d. Effects of pregnancy, *V. B.* (L.M.P. 6/5/39, graphs 8/4/39): 8*a*, right breast; 8*b*, left breast; 8c, right hand; 8d, left hand.

Fig. 9*a*, 9*b*, 9c and 9d. Effects of pregnancy, 5 mo., *V. B.* (11/30/39): 9*a*, right breast; 9*b*, left breast; 9c, right hand; 9d, left hand.

Fig. 10*a*, 10*b*, 10c and 10d. Records of breast pathology: 10*a*, over adjacent area of left breast (*M. M.*); 10*b*, over cyst of left breast (*M. M.*); 10c, over adjacent area of right breast (*R. J.*); 10d, over fibroadenoma right breast (*R. J.*).

throughout the study. *V. B.*'s records showed no such distinctive differences between the right and left breast curves at any time. Figure 4, showing specimens from records taken of male hands, exhibits a striking uniformity of the wave in all the graphs on this subject (*M. B. J.*).

The manner in which breast curves are influenced by drugs of known action upon the peripheral circulation has been studied. These records show changes in the shape of the curves, as well as in amplitude and width. Figure 5 shows the effects of amyl nitrite and figure 6 of tobacco. Figure 7 was obtained upon constriction of the breast by means of an elastic band. Figures 8 and 9 show records obtained during early and late pregnancy.

At present we interpret these findings as follows. At the onset of menstruation

there is generalized vasodilation, resulting in a rounded wave without the dicrotic notch in the breast plethysmographs and a faint notching in hand plethysmographs; during pregnancy there is persistent vasodilation in the breasts without the same effects upon the general circulation.

While these studies are entirely qualitative, only the wave form being considered, quantitation seems feasible and is now being attempted.

Graphs taken over a cyst of the breast and over a fibroadenoma of the breast show notable differences in the shape of the curves as compared with the adjacent tissues of the same breasts (fig. 10).

From our small series, it cannot be claimed that these curves are characteristic or typical. As preliminary studies they seem to us encouraging, and suggest several directions for further investigations.

SUMMARY

An improved photoelectric plethysmograph is described. Specimens of daily breast records in normal females indicate the diurnal variability of female breast circulation. There is less variation in the wave-form registered by transillumination of the web of the hand in the same subjects. Studies on male hands show striking uniformity in the shape of the graphs.

Breasts records made during pregnancy show the wave form associated with the onset of menstruation; in plethysmographs of the hands of these subjects the intermenstrual type of curves are obtained.

Characteristic changes in graphs of female breast circulation produced by amyl nitrite administration, smoking, and constriction of the breast, are shown.

Notable differences in the size and shape of curves were obtained by transilluminating a cyst and a fibroadenoma of the breast as compared with records obtained through adjacent areas of the same breasts, and with each other.

We wish to acknowledge the valuable suggestions and technical aid of Dr. Hubert Mann; Dr. Alexander Karlin, physicist to the Mt. Sinai Hospital, New York; and to Mr. Winfield Nichols, who constructed our apparatus.

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FRACTIONATION OF SALINE EXTRACTS OF ANTERIOR PITUITARY TISSUE[1]

ROY W. BONSNES[2] AND ABRAHAM WHITE

From the Laboratory of Physiological Chemistry, Yale University School of Medicine

NEW HAVEN, CONNECTICUT

A CONSIDERATION of the literature (1)[3] dealing with the extraction and purification of the hormones of the anterior pituitary makes it evident that active preparations of the growth, adrenotropic, thyrotropic, gonadotropic and lactogenic factors have been obtained. Moreover, some degree of purification of each of these protein hormones has been accomplished by the removal of other proteins.

In addition to the above-mentioned hormonal factors, preparations of one or more of the various metabolic hormones of the anterior pituitary have also been described. The methods employed for the preparation of these hormones and the properties of the products obtained indicate that these substances are proteins or protein-like in composition. The hormones may be extracted from the gland by aqueous, acid, or alkaline solutions. They may be precipitated by alterations in hydrogen ion concentration, by organic solvents or by inorganic salts. Further evidence that the anterior pituitary hormones are proteins is obtained from the observation that the physiological activity of some of these substances is destroyed by heat, by strong acids and alkalies, and by proteolytic enzymes.

The many instances in protein chemistry in which saline media have been employed for the extraction of proteins from plant and animal tissues suggested a study of saline technics as a means for obtaining preparations of the anterior pituitary hormones. Efforts were directed towards evolving a procedure giving a saline extract containing as many as possible of the physiological activities assigned to the anterior pituitary. It was desired to fractionate the extract with the view of obtaining the various factors in as pure a form as possible. It was considered advisable to avoid, if possible, specialized technics of the type of adsorption or the use of non-specific protein precipitating agents, like sulfosalicylic or picric acids. Time-consuming procedures such as dialysis, or the concentration of large volumes at low temperatures, were also considered undesirable. The simple use of alterations in hydrogen ion concentration, with the subsequent addition of acetone to remove more soluble fractions was evolved as the most convenient method of fractionation.

Crude saline extracts have previously been employed as a source of physiologically active pituitary fractions. This type of extract was early used by Evans and Long (3) to demon-

[1] This investigation was aided by grants (to A. W.) from the Committee on Therapeutics, Council on Chemistry and Pharmacy, American Medical Association, and the Yale University School of Medicine Fluid Research Fund, and by a grant to Dr. C. N. H. Long from the Committee on Research in Endocrinology, National Research Council.

[2] Some of the data in this paper are taken from a Dissertation presented by Roy W. Bonsnes as partial fulfillment of the requirements for the degree of Doctor of Philosophy, Yale University.

[3] Adequate reference to the literature cannot be made because of the limitation of space. The recent monograph by Van Dyke (1) presents a complete bibliography and review. Reference may also be made to the *Symposia on Quantitative Biology* (2).

strate the growth promoting activity of the anterior pituitary. Long and his collaborators (4), in this laboratory, have utilized crude saline AP extracts as a source of the metabolic hormones, and similar extracts have been employed by Young (5) in his studies of the diabetogenic and glycotropic activities of the anterior pituitary. The latter investigator has recently presented (6) a study of the fractionation of saline diabetogenic extracts of ox anterior pituitaries by fractionation with ammonium sulfate at 0° C. By adjusting the hydrogen ion concentration of a saline extract to pH 5.0 to 5.4, Young effected a separation of prolactin. The filtrate from the latter hormone was then separated into 'globulin,' 'pseudoglobulin,' 'euglobulin,' and 'albumin' fractions by increasing concentrations of ammonium sulfate, and a study made of the physiological and chemical properties of the fractions obtained. Saline extracts of hog pituitary have been used as initial material in a study of the action of proteolytic enzymes on some of the hormones of the anterior lobe (7).

EXPERIMENTAL

Fractionation procedure. Anterior lobes dissected from fresh beef pituitary glands, frozen at the slaughter house, have been used throughout this investigation. Extractions have been conducted with 100, 200, and 300 gm. quantities of tissue, and the fractionation procedures have yielded the same type of products, regardless of the quantity of material extracted. The amounts of each fraction obtained are, of course, dependent on the initial amount of glands taken; the yields stated in the procedure below are those observed when 100 gm. of anterior lobes are extracted. After many preliminary studies, the following fractionation procedure was evolved.

Anterior pituitary lobes (100 gm.) are thoroughly ground in a small meat grinder and mixed with 500 cc. of a 2% NaCl solution. The mixture is immediately adjusted to pH 7.4 to 7.8 by the addition of a suitable amount of 2N NaOH.[4] One to two cc. of the alkali is required for each 100 gm. of tissue. Extraction is conducted at room temperature for 2 hours with the aid of mechanical stirring. If necessary, small quantities of 2N NaOH are added at intervals in order to maintain the pH between 7.4 and 7.8. The pH of the mixture may be conveniently tested at half-hour periods. The mixture is then centrifuged and the turbid supernatant further clarified with the aid of a Sharples supercentrifuge. The clear, reddish solution is then adjusted to pH 5.4 and allowed to stand in the ice box overnight. The white precipitate which settles out is removed by centrifugation. This fraction, and all subsequent precipitates, are obtained as dry powders by washing with acetone 3 times at the centrifuge and drying in a vacuum desiccator over concentrated sulfuric acid. The precipitate obtained at pH 5.4 has been called the 'A fraction.' The range of yields of the A fractions has been 0.7 to 1.4 gm. After removal of the A fraction, the solution is adjusted to pH 4.8. The white flocculent precipitate which settles is allowed to stand in the ice box overnight and then removed by centrifugation (*B* fraction; range of yields = 0.3 to 2.0 gm.). The clear red supernatant is then adjusted to pH 4.1 and the red-brown precipitate which forms is removed, without further standing, by centrifugation[5] (fraction *BA*; range of yields =0.7 to 1.4 gm.). An equal volume of acetone is added to the yellow solution remaining after removal of the *BA* fraction. On standing overnight in the ice box, a precipitate forms. This precipitate is removed by centrifugation (fraction C; range of yields =0.2 to 0.7 gm.). Increasing the acetone concentration of the supernatant to 75% by volume gives another white precipitate which may be separated by centrifugation (fraction *CA*; range of yields =0.3 to 0.8 gm.). If the acetone concentration is then brought to 90%, a light brown precipitate can be obtained which gives a strongly positive biuret reaction (fraction *D*; range of yields =0.4 to 0.6 gm. This fraction contains

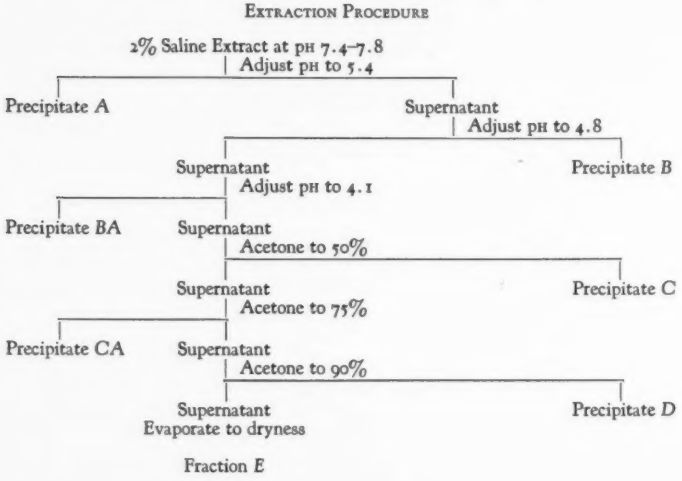
[4] All pH determinations have been made with the glass electrode.

[5] The blood pigments in the extract will precipitate with this fraction (*BA*) and occasionally with the following fraction (C). The color in the precipitates is generally completely removed by treatment with acetone, and a grey to colorless powder is obtained. If a period of time is allowed to elapse before removal and washing of the precipitates by centrifugation, considerable difficulty may be encountered in efforts to completely decolorize these fractions.

approximately 14% ash). Finally, concentration of the supernatant from fraction *D* to dryness in vacuo at low temperatures yields a brown residue which, when thoroughly dried, contains approximately 25% organic solids (fraction *E*; yield, approximately 7 gm. of total solids). The general steps in the extraction procedure are outlined in the diagram.

Properties of fractions. All of the fractions which have been obtained are protein or protein-like in their properties. Fractions *D* and *E* have not been examined extensively because, as will be described in a later section, neither of these fractions exhibited any of the hormonal activities for which tests were conducted. These two fractions are very soluble in water and yield solutions giving positive biuret reactions.

The other fractions give the usual protein color reactions: the biuret, xanthoproteic, Hopkins-Cole and Millon's reactions are all positive. Each of these protein frac-



tions is precipitated from solution by phosphotungstic or sulfosalicylic acids. Fractions *A*, *B* and *BA* are insoluble in water but dissolve readily on addition of dilute (0.1 N) sodium hydroxide, provided the dry material is thoroughly dispersed in water before addition of the alkali. The proteins may then be precipitated from the alkaline solutions by careful addition of dilute HCl to the pH values at which these fractions were originally precipitated. Fractions *C* and *CA* are partially soluble in water and this fact has been made the basis for further purification studies of the *CA* fraction. The water-soluble material is readily precipitated by the addition of several volumes of acetone. Further properties of the individual fractions, including measurements of homogeneity and molecular size, will be described in subsequent publications.

Bioassay methods used. Prolactin activity was determined by the 2-day 'local' crop method of Lyons and Page (8), using immature pigeons.

Thyrotropic assays were conducted on 1-day-old White Leghorn chicks. The histological changes, rather than weight increases, in the thyroid glands were used as an index of the activity of the preparations, inasmuch as definite histological alterations could be observed in some instances in which there were no changes in weight of the thyroids and, in some animals, weight increases were obtained in the thyroids without definite histological changes.

Otherwise, the technic of the assay is that described by Smelser (9); daily injections were given for a period of 5 days with autopsy 24 hours following the last injection.

The growth activity of the preparations was determined on hypophysectomized rats weighing between 93 and 115 gm. Only animals which failed to gain weight for a period of 3 weeks following hypophysectomy were used for the assays of growth potency. The growth activity is judged from the average daily gain in weight over a period of 10 days. The preparations tested were administered in solution in daily, single intraperitoneal injections.

The adrenotropic activity was determined on 21-day-old rats as described by Moon (10). Better responses were obtained if the material was given in 3 doses daily for 3 days instead of in single daily injections as described by Moon.

Diabetogenic activity was determined by giving a single subcutaneous injection of a preparation, in solution, to a partially depancreatized rat which has previously been demonstrated

TABLE 1. DISTRIBUTION OF ACTIVITIES OF FRACTIONS OBTAINED FROM SALINE EXTRACT

Type of Activity	Fraction						
	Saline	*A*	*B*	*BA*	*C*	*CA*	*D*
Prolactin	+	+	±	o (100γ)	o (1000γ)	o (1000γ)	o (1000γ)
Thyrotropic	+	o (5 mg.)	o (5 mg.)	±	±	+	±
Growth[1]	+	o (100γ)	+	+	o (100γ)	o (100γ)	o (100γ)
Adrenotropic	+	+	±	o (60 mg.)	o (60 mg.)	o (60 mg.)	o (60 mg.)
Diabetogenic	+	+	+	+	+	o (5 mg.)	o (5 mg.)
Glycostatic	+	not assayed	+	±	+	+	not assayed

+, Present; ±, trace; o, no response at the dose level indicated in parentheses.

[1] Assays for growth promoting activity were conducted on fractions which had each been reprecipitated 3 times, preliminary to further purification studies. Fractions *A*, *B* and *BA* were reprecipitated by suspending the proteins in water and then adding dilute alkali to effect solution (PH 8–10). The material was then precipitated by adjusting the solution to the isoelectric point of that particular fraction. Fractions *C*, *CA* and *D* were reprecipitated by dissolving the proteins in water and precipitating again with acetone at the same concentration as was used for their initial isolation from the saline extract. The small amounts of water-insoluble material were not separated prior to the acetone addition.

to be responsive (glycosuria initiated or augmented) when injected with crude saline extracts of anterior pituitary tissue. The method is not quantitative and will only indicate the presence of diabetogenic activity (11). A positive response is evidenced by a definite glycosuria appearing on the day following the injection or by an increased urinary glucose in rats previously exhibiting a spontaneous glycosuria.

Glycostatic activity has been determined in some instances. These assays were kindly conducted by Dr. Jane Russell by a procedure already described (12).

Gonadotropic activity has not been extensively investigated. The original 2% saline extract exhibited a slight gonadotropic potency when assayed on 21-day-old female mice. However, the gonadotropic response obtained with the saline extract was so weak when compared with active gonadotropic preparations that further studies of the separation of gonadotropic activity from the saline extracts were not conducted.

Results of bioassays. Throughout this investigation no effort has been made to express the various types of biological potencies in terms of units or as related to degree of concentration of activity which has been effected by the fractionation procedure employed. Rather, as already stated, it has been considered primarily desirable to separate as many of the anterior pituitary factors as possible before attempting purification or further concentration experiments. In most instances, therefore, the bioassays were conducted with the view of determining *a*), the presence or absence of significant amounts of a given pituitary hormone in a preparation, and *b*), if present, the minimum effective dose of a preparation, i.e., the minimum quantity of a preparation which will produce an unquestionably positive response under the conditions of the bioassay used. The pertinent data of the bioassays are summarized in table 1. Table 2 indicates in more detail the dose levels at which the various potent fractions were effective.

TABLE 2. BIOASSAYS OF FRACTIONS FROM 2% SALINE EXTRACTS

Type of Activity	Preparation	Dose Level[1]	Remarks
Prolactin	Saline extract	25–50γ	M.E.D.[2]
	A	1– 5γ	M.E.D.
	B	1000γ	Questionable response at times, usually negative
Thyrotropic	Saline extract	100γ	M.E.D.
	BA	500γ	M.E.D.
	C	200γ	M.E.D.
	CA	50γ	M.E.D.
	D	500γ	M.E.D.
Growth	Saline extract	100γ/day	1.5 gm. av. gain/day
	B	100γ/day	0.9 gm. av. gain/day
	BA	100γ/day	0.9 gm. av. gain/day
Adrenotropic	Saline extract	60 mg.	44% increase in adrenal weight over controls
	A	60 mg.	44% increase in adrenal weight over controls
	B	60 mg.	12% increase in adrenal weight over controls
Glycostatic	Saline extract	1.7 mg./100 gm. rat	1 U[3]
	B	1.6–1.9 mg./100 gm. rat	1 U
	BA	5.5 mg./100 gm. rat	1 U
	C	1.2–2.5 mg./100 gm. rat	1 U
	CA	1.5 mg./100 gm. rat	1 U
Diabetogenic	The saline extract and fractions *A*, *B*, *BA* and *C* each produced a definite glycosuria in the partially depancreatized rat at levels of 3 to 5 mg./100 gm. rat.		

[1] Total dose in terms of total organic solids unless otherwise noted.
[2] M.E.D.—minimum effective dose.
[3] See Russell (12) for definition of glycostatic unit (U).

DISCUSSION

It is not desired to imply that the preparations which have been obtained by the described fractionation procedure are individual proteins. The necessary experiments for establishing the homogeneity of these products have not as yet been conducted. However, the ready repetition of this procedure and the similarity in yields and in types of products in each complete fractionation which has been conducted are en-

couraging for orientation experiments. Moreover, under the conditions of this saline procedure, the adjustment of the extract to pH 5.4 gives a flocculent precipitate which settles rapidly and leaves a clear supernatant solution. At pH values of ± 0.2 pH units from 5.4, precipitates are obtained but the supernatant solutions are generally turbid. This observation would indicate that the bulk of the protein precipitated at this point is a single entity or that two or more proteins are present which exhibit minimum solubility at exactly the same hydrogen ion concentration. Similar observations have been made with respect to the *B* (pH 4.8) and the *BA* (pH 4.1) fractions. The relative homogeneity of the *A* and *B* fractions has already been demonstrated by electrophoretic studies (13).

On the other hand, the fractions which are obtained by precipitation with acetone are not as clearly defined. The *C* fraction probably contains some portion of fractions *A*, *B* and *BA*, each of which may be slightly soluble at the hydrogen ion concentrations at which they appear to precipitate most readily. The *CA* fraction, however, does flocculate readily when the acetone concentration is brought to between 70 and 75%.

Fractions *D* and *E* both contain considerable amounts of inorganic salts and may again represent protein material which has not been completely removed in previous steps. There may also be present in these fractions molecules which, although they give a positive biuret reaction, are smaller in size than the proteins commonly encountered in biological extracts. This latter conclusion is tentatively based on the solubility of these fractions in high concentrations of acetone and on the delicate, light pink color obtained in the biuret test.

The results of the bioassays demonstrate that the prolactin activity is uniformly found in the *A* fraction. In some fractionation experiments prolactin potency was also present in *B* fractions, but this has not been a consistent finding inasmuch as most *B* fractions yielded negative results when tested at 100-gamma levels, while in several instances no prolactin response was obtained when this fraction was assayed at a level of 1000 gamma. The occasional presence of prolactin activity in a *B* fraction is not surprising in view of the small difference in hydrogen ion concentration between the points at which the *A* and *B* fractions are removed. Furthermore, it should be kept in mind that a large portion of the bioassay data which are presented have been obtained on fractions precipitated directly from the saline extract and subsequently washed with acetone. The presence of mother liquor in the precipitates might therefore be expected, and overlapping of activities in the various preparations is not unlikely. Complete separations are being sought in experiments being conducted at the present time. It may also be noted that there has been some concentration of prolactin in the *A* fraction since this precipitate shows at least a 5-fold increase in potency over the original extract.

The assays for thyrotropic activity indicate that there is a considerable concentration of this hormone in the *C* and *CA* fractions. It appears evident that this type of activity is not present to any significant extent in the *A* and *B* fractions. This definite restriction of thyroid-stimulating substance to a few fractions is particularly noteworthy inasmuch as a relatively small amount of this hormone is required to produce a positive histological response in the thyroid of the day-old chick.

The growth activity assays indicate that the material which promotes growth in hypophysectomized rats is found in the *B* and *BA* fractions and not in the *A*, *C*, *CA* and *D* fractions. However, it should be noted that reprecipitated *B* and *BA* preparations had, insofar as the accuracy of the assay method permits one to judge, an appar-

ently somewhat smaller degree of growth promoting activity when compared to the original extract. Efforts are being made to establish whether or not this is a real difference in potency and to determine whether the growth hormone is a protein which precipitates in a given range of hydrogen ion concentration or whether it is a smaller molecule which exhibits optimum adsorption tendencies at certain pH values. Evans, Meyer and Simpson (14) have reported a gradual diminution in growth promoting activity following repeated isoelectric precipitations of active preparations.

The method of assay for adrenotropic activity has not yielded uniform results throughout this investigation, although better responses are obtained when injections are given 3 times daily, rather than singly, on each of 3 successive days. However, wide variation in adrenal weights has been observed in animals of a fairly uniform body weight. Insofar as it is possible to base any reliance on the assay method employed it appears that there has been some separation of adrenotropic activity. The average increases in adrenal weights produced by fractions *A* and *B* is greater than the adrenal weight increases observed in uninjected, control animals. The average adrenal weights obtained in animals receiving fractions *BA*, *C*, or *D* were not significantly higher than those of the control animals. There has not, however, been any degree of concentration of the adrenotropic factor, even though some separation has been effected. The original saline extract, in terms of total organic solids, gives the same increase in adrenal weights as that produced by fraction *A*.

The diabetogenic activity of the reprecipitated fractions permits only the conclusion that this type of physiological activity is present in all but the *CA* and the *D* fractions. The diabetogenic assay suffers from variability and lack of sensitivity, and it is not readily possible to determine accuractly the minimum effective dose for each preparation. Preliminary experiments have been conducted in which determinations of ketogenic activity, as measured by increase in the level of blood ketones of the fasting, normal rat (11), has been studied and correlated with diabetogenic potency. The results available at the present time would appear to indicate that ketogenic activity is concentrated chiefly in the *B* fraction. Further purification of the latter preparation is being conducted. It may be noted that the recent data of Young (6) suggest that diabetogenic activity, as determined by the ability of preparations, on repeated injection, to produce glycosuria in normal dogs, is concentrated in the 'globulin' and 'pseudoglobulin' fractions of his saline extracts. In the present study, fractions *A*, *B* and *BA* exhibit the properties of globulins, as judged by their solubility behavior, and these fractions also exhibited diabetogenic activity in the partially depancreatized rat. However, the diabetogenically active products of Young also possessed thyroid stimulating properties, whereas the *A* and *B* fractions which have been obtained in the fractionation described in this paper did not give histological thyroid responses in the day-old chick when assayed at relatively high dose levels. Young has emphasized that preparations which are thyrotropic are not necessarily diabetogenic, and this conclusion is in agreement with the present data inasmuch as fraction *CA*, which exhibited marked thyrotropic activity, did not give a diabetogenic response under the conditions of assay employed.

Glycostatic activity has been found to be present in fractions *B*, *C* and *CA;* these fractions have a glycostatic potency approximating that of the original saline extract.

It has thus been demonstrated in the present study that a 2% saline extract of the anterior pituitary contains many of the physiological properties attributed to this gland. Bioassays of these fractions indicate that, in general, there have been no absolute separations of the various biological activities nor was there any apparent con-

centration of potency, except in the cases of prolactin and the thyrotropic hormone. In view of these results it appears likely that the proteins which are obtained by the methods described in the present study are chiefly inert tissue proteins which carry with them relatively small amounts of potent hormones. A similar conclusion has been drawn from an investigation of the electrophoresis of anterior pituitary proteins (13). The evidence at hand indicates that the actual concentration of the protein hormones present in anterior pituitary tissue, compared to the amount of structural tissue protein, is very small indeed. In view of these facts it is not surprising that a considerable overlapping of the various types of physiological activities has been observed in the protein preparations. However, it is encouraging to note that in a few instances some degree of purification of the hormones has been attained by this relatively simple fractionation procedure. A product has been obtained which possesses a relatively good degree of lactogenic potency, and another preparation has been obtained which has a marked thyroid stimulating effect. Certain of the other types of hormones have also been segregated adequately to encourage the further purification studies which are in progress.

Differences in the methods which have been employed by investigators in the assay of anterior pituitary preparations make it difficult to compare quantitatively the potency of the various products obtained with those described in the literature. It can be said that the lactogenic fraction (fraction A) obtained in the present study is definitely less potent than the purified prolactin yielded by other methods (15, 16, 17). Moreover, the contamination of the A fraction with tissue proteins makes the products described in the literature (15, 16, 17) more desirable as a source material for prolactin investigations. The growth promoting and thyrotropic activities of the preparations which have been obtained in these experiments are, however, of a great enough magnitude in certain of the fractions to make the latter compare favorably with the growth and thyrotropic preparations from other laboratories. The partially purified growth hormone obtained by Evans, Uyei, Bartz and Simpson (18), when tested in hypophysectomized rats at a dose level of 90 γ daily, induced a gain in weight of 14 gm. in 10 days. The highly purified preparations which have been described (18, 19), however, appear to exhibit a greater growth promoting potency than the fractions obtained in the present study. The thyrotropic activity of fraction CA compares very favorably with the preparation described by Smelser (9). The product of the latter investigator produced a histological response and a questionable weight increase in the thyroids of day-old chicks when administered in a total dose (over 5 days) of 100 γ.

SUMMARY

A procedure is described for the fractionation of a 2% saline extract (pH 7.4 to 7.8) of the anterior lobes of beef pituitaries. Using this technic, it has been possible to obtain 6 fractions which exhibit the properties of proteins. Bioassays have been conducted for the lactogenic, thyrotropic, diabetogenic, glycostatic, adrenotropic and growth promoting activities of most of these fractions.

It has been demonstrated that a significant separation of each of the following types of anterior pituitary activities has been achieved: lactogenic, thyrotropic, growth promoting and adrenotropic.

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STUDIES IN THE PHYSICAL CHEMISTRY OF THE ANTERIOR PITUITARY HORMONES

SEPARATION OF FIVE ANTERIOR PITUITARY HORMONES INTO DIFFERENT FRACTIONS BY ISOELECTRIC AND AMMONIUM SULFATE PRECIPITATION[1]

H. L. FEVOLD, MILTON LEE, F. L. HISAW AND E. J. COHN

From the Departments of Physical Chemistry and Physiology, and the Memorial Foundation for Neuro-Endocrine Research in the Harvard Medical School, and the Biological Laboratories, Harvard University

BOSTON AND CAMBRIDGE, MASSACHUSETTS

THE ISOLATION as chemical individuals of the various proteins of any tissue system would appear to be not only possible but desirable to the end of understanding in how far the physiological functions ascribed to the tissue are attributes of different molecules. The anterior pituitary gland has been chosen for the present study since it has been shown to subserve a wide variety of physiological functions. There has been considerable discussion as to whether or not these functions are performed by the same or by different molecules. Many of the anterior pituitary hormones are proteins and the problem of their separation is therefore one of protein chemistry. It seemed desirable to determine how far the methods that have been developed for the separation of the serum and other proteins (1, 2) could be employed in the fractionation of the anterior pituitary gland.

Five of the hormones, the two gonadotropins (FSH and LH), thyrotropin, adrenotropin and the growth hormone, were chosen for investigation. Procedures for the assay of these are relatively satisfactory and could readily be carried out in one or another of our laboratories. These five hormones have been extracted from the raw glands and separated into different protein fractions with little or no loss in yield. Study of the other proteins has been postponed for a later time. The chemical methods that have been employed were chosen because they are believed to preserve most of the cell proteins unaltered, thus making it possible subsequently to recover and purify also certain of the other hormones not considered in this communication. Methods employed in the bioassays of these hormones are given below.

METHODS OF BIOLOGICAL ASSAY

Luteinizing hormone. The luteinizing hormone was assayed by a method previously described (3). Immature male rats, 21 days old, were injected intraperitoneally, twice daily, for 4 days and the seminal vesicles weighed on the morning of the fourth day. The smallest amount of the material to produce 100% increase in weight over those of uninjected controls is a rat unit.

Follicle stimulating hormone. This method has also been previously described (4). The amount necessary to cause 100% increase in the ovarian weights of immature female rats, when injected subcutaneously, twice daily, for 4 days is defined as the rat unit.

[1] Aided by a grant from the Rockefeller Foundation.

Adrenotropic hormone. The method used was that reported by Moon (5). The minimal amount necessary to produce 100% increase in the adrenal weights of 8-day-old rats injected intraperitoneally, once daily, from the fourth to the eighth day of life, was considered a unit.

Thyrotropic hormone. This hormone was assayed in young male chicks according to the method reported by Smelser (6), and by Bergman and Turner (7). One-day-old chicks were injected, once daily, for 4 days and the thyroid dissected out and weighed. The minimal amount necessary to cause 50% increase in thyroid weight is the unit used in this paper.

Growth hormone. Growth fractions were assayed by a method described elsewhere (8), using hypophysectomized rats in groups of 15 to 20. The extract was injected subcutaneously once daily, for 3 days. The potency was determined from a 'characteristic' curve based upon percentage gain in body weight. Roughly, 1 unit will cause an increment of 3.4% in body weight in 66 hours.

METHOD OF CHEMICAL FRACTIONATION

Extraction at pH 8. Whole pituitary glands of sheep are frozen, thawed, refrozen with CO_2 snow and finely ground in order to permit more quantitative extraction. This procedure is carried out in the cold, at pH 8.0, using 2 liters of dilute aqueous ammonium hydroxide per kg. of gland. The gland residue, separated by centrifugation from the extractives, is reextracted with 2 liters of solution containing sufficient dilute aqueous ammonium hydroxide to maintain the pH at 8.0. These extracts are combined. The crude alkaline extract has not yet been satisfactorily assayed for FSH because of interference with the assay by the other gonadotropic hormone. In the case of the growth, luteinizing, thyrotropic and adrenotropic hormones, however, our results indicate that essentially all of each hormone has been extracted.

Separation of precipitate at pH 5.4. Observations previously reported indicate that a fair proportion of the total extractives are precipitated at reactions near pH 5 (9–16). The supernatant solution from our pH 5.4 precipitate, although somewhat reddish in color, was water-clear and satisfactory for further fractionation. It has also been suggested that the growth hormone is euglobulin in nature and is isoelectric near pH 4.8. In our preliminary studies, the growth hormone was in part precipitated when the pH 8.0 extract was brought to pH 5.4. In part it remained in solution because of the salt accumulated by the neutralization.

In order to avoid having the growth hormone in two fractions, the combined pH 8.0 alkaline extracts are rendered quarter molar with respect to ammonium sulfate before being brought to pH 5.4 with N/10 sulfuric acid. The growth hormone is less stable in acid than in alkaline solution (9, 11). For this reason precipitation is carried out at pH 5.4 rather than at the reported isoelectric point of about 4.8 (14). The precipitate settles satisfactorily. As a further precaution the precipitate is separated from the 0.25M ammonium sulfate filtrate in the cold and the supernatant returned as rapidly as possible to pH 7.0. The precipitate is redissolved at pH 8.0 and reprecipitated as before. When the precipitation was carried out in this way in the presence of 0.25M $(NH_4)_2SO_4$ it was found that all of the growth hormone remained in the filtrate.

This pH 5.4 precipitate contains the adrenotropic hormone. Our assays for adrenotropin in the pH 5.4 precipitate showed 5000 R.U. per kg. of gland. None remained in the 0.25M $(NH_4)_2SO_4$ solution.

Values on 4 preparations are given in table 1 for the solids extracted, and the division of the solids in the pH 5.4 soluble and insoluble fractions. The various yields of the hormones found in each fraction are also given. The values shown for FSH are only rough approximations since this hormone cannot be determined quantitatively in the presence of LH.

Precipitation with ammonium sulfate. Approximately 23 gm. of protein per kg. of frozen gland remains in the 0.25M $(NH_4)_2SO_4$ solution which is brought to pH 7.0.

The dry weight of the frozen gland is approximately 200 gm. per kg., of which from 58 to 83 gm. have been extracted at pH 8.0 in our various preparations. Just over 1/10 of the solids of the total gland, and from 1/3 to 1/4 of the total extractives, remain in solution at pH 5.4. This fraction contains the growth hormone, thyrotropin and both gonadotropins. The growth hormone could be separated from this solution by dialyzing it salt-free, since it alone of these hormones appears to be a euglobulin.[2]

TABLE 1. EXTRACTION OF ANTERIOR PITUITARY HORMONES

	Preparation	Solids[1]	Adrenotropic hormone	Growth hormone	Luteinizing hormone	Thyrotropic hormone	Follicle stimulating hormone[2]
		gm./kg.	R.U./kg.	R.U./kg.	R.U./kg.	C.U./kg.	R.U./kg.
pH 8.0 Extract	III	59.8		50,000	8,000		(2500)
	IV	58.0		52,000	12,000	2000	(3000)
	V	82.5	5000	52,000	18,000	3000	(2000)
	VII	71.2	5000	50,000	20,000	4000	(3000)
pH 5.4 Precipitate, in 0.25M $(NH_4)_2SO_4$	III	40.4			none	none	none
	IV	35.4	5000	none	none	none	none
	V	59.4	5000	none	none	none	none
	VII	48.1	5000	none	none	none	none
pH 5.4 Solution, in 0.25M $(NH_4)_2SO_4$	III	19.4			8,000		(2500)
	IV	22.6	none	76,000	14,000	2000	(3000)
	V	22.9	none	112,000	16,000	3000	(2000)
	VII	23.8	none		20,000	4000	(3000)

[1] All solids were determined by dry weight of aliquots after dialysis. Assay units are per kg. of fresh gland.

[2] Values given are only approximations, calculated from the number of units of FSH recovered after separation from LH. No method is available for the accurate determination of FSH in the presence of LH.

The volume of the combined extracts is large at this point in our procedure, and it is more satisfactory first to separate both the water-soluble and salt-soluble proteins by fractional precipitation with $(NH_4)_2SO_4$, removing the euglobulin by subsequent dialysis of each precipitate.

During the ammonium sulfate precipitation the pH is maintained at approximately 7.0. The pH throughout our procedure has been retained between 5.4 and 8.0 and the only ions[3] which have been added are ammonium and sulfate.

The theory of the solubility and the interactions in systems containing more than one protein component suggests the desirability of carrying out protein fractionations in such a manner as to avoid local excess of any reagent. The procedure employed throughout this investigation has been that described by McMeekin (1) for the precipitation and purification of serum proteins. The ammonium sulfate was added very slowly by diffusion through a rotating cellophane membrane which stirred the protein solution. Smaller molecules such as the posterior pituitary hormones presumably pass through the membrane into the more dilute $(NH_4)_2SO_4$ solutions, from which they could be recovered by precipitation through the addition of more $(NH_4)_2SO_4$.

In the further separation of the protein hormones of the pituitary gland more concentrated $(NH_4)_2SO_4$ is allowed to diffuse slowly into the 0.25M $(NH_4)_2SO_4$ solution containing the extracted albumins and globulins. The pH is maintained at or near 7.0

[2] Since this was written, assays for the lactogenic hormone have demonstrated that it also is a euglobulin, extracted by 0.25M $(NH_4)_2SO_4$ solution and precipitated both by higher concentrations of $(NH_4)_2SO_4$ and by dialysis. The suggestion of Collip that this and the growth hormones are closely related chemically is thus confirmed by our observations.

[3] Other than a slight amount of carbonate derived from the CO_2 snow used in freezing the glands.

by the addition from time to time of small amounts of 1% NH_4OH solution, the whole process being carried out in the cold. No appreciable precipitate appears before the protein solution is rendered more than 1.0 molar. From 1.0M to 1.4M a fairly large fraction of protein precipitates. This is separated by centrifugation. Thereafter the fractions separating at small increments of ammonium sulfate concentration are separately removed. Since these precipitates contain both water-soluble and salt-soluble proteins each fraction is dialyzed separately against running cold distilled water saturated with toluol. The euglobulins separating upon dialysis are centrifuged and repeatedly washed with small amounts of cold distilled water, the washings being combined with the filtrates.

Growth hormone. More than half of all the globulins present are precipitated by 1.6M ammonium sulfate and all but small amounts are precipitated by 2M (0.5 saturated). The growth hormone is largely precipitated by 1.6M and completely precipitated by 1.8M ammonium sulfate, as well as by dialysis. Our results thus confirm those of Teel (17), who reported that the growth hormone is completely precipitated by 1.4M sodium sulfate, and that the globulin fraction precipitating between 1.4 and 2.5M sodium sulfate is inactive.

In further purification the growth fraction precipitated by ammonium sulfate is freed of salt by dialysis and the globulins precipitated. The precipitate contains a certain amount of protein besides that readily soluble in 0.25M $(NH_4)_2SO_4$ at pH 7.0. This protein is presumably comparable to that precipitated by pH 5.4 but which had remained in solution because of the large volume employed in the original extraction (5 liters per kg. of gland). The precipitate derived from each kg. of gland is therefore extracted twice with 500 cc. of 0.25M $(NH_4)_2SO_4$ at approximately pH 7.0. This solution is adjusted to pH 5.4 with N/10 H_2SO_4, the precipitate quickly separated in the cold by centrifugation and the solution again brought to pH 7. The precipitate thus removed has shown no growth activity.

The globulins are again precipitated by the slow addition of ammonium sulfate according to McMeekin's method. The precipitate is collected by centrifugation and redissolved in a still smaller volume (100 cc. per kg. of gland) of 0.25M $(NH_4)_2SO_4$. Each decrease in volume led to a further precipitation of the proteins insoluble at pH 5.4 and these have been removed during the successive purifications. The purified material per kg. of fresh gland weighed 0.44 gm., and assayed 51,000 U of growth hormone, or 115 U per mg.

Luteinizing hormone. The range over which the luteinizing hormone is precipitated by ammonium sulfate at pH 7.0 appears to be from 1.4 to 2.4M. The greatest portion of the activity has, however, been recovered in the fraction separating from 1.6 to 2M. This confirms results previously reported (18, 19). The activity of the precipitate in three different experiments over this range represented more than three-fourths of that extracted. Smaller amounts have been recovered from the proteins precipitated at 1.6M and those remaining in solution at 2M. The luteinizing hormone is water-soluble and its separation from the growth hormone was accomplished by dialysis. These crude LH fractions contain from 5 to 10 R.U. per mg. of material.

In further purification of LH, the fractions are combined and dissolved in 100 cc. of water per kg. of gland. Upon reprecipitation with $(NH_4)_2SO_4$, added slowly through a cellophane membrane, the material insoluble at 1.5M is separated, redissolved and reprecipitated 4 times, after which it is free of LH. The combined 1.5M $(NH_4)_2SO_4$ solutions are then brought to 2.4M $(NH_4)_2SO_4$ to insure complete precipitation. This precipitate contains 30 to 40 R.U. per mg. of material and contains none of the other pituitary hormones for which tests have been made.

Thyrotropic hormone. All of the thyrotropic hormone has always been found in

the water-soluble extractives. None has been precipitated at pH 5.4 or by dialysis. Only traces of it have been precipitated in the fractions less concentrated in ammonium sulfate than 2M at the volume of our preliminary 'salting out.' Although complete recovery has not yet been obtained in the case of this hormone, it appears to have been entirely precipitated when the ammonium sulfate concentration reached 2.4M.

It has recently been suggested (19) that the luteinizing and thyrotropic action of pituitary extracts may be carried out by a single factor. The experiments here reported do not support this contention but indicate that the two effects are due to separate hormones.

Follicle stimulating hormone. The FSH is the most soluble of the anterior pituitary hormones here considered. Only traces of this factor were precipitated from our original extract by $(NH_4)_2SO_4$ at a concentration of 2.4M and near pH 7.0. At 2.8M the precipitation appeared to be complete. Assay revealed no FSH in the supernatant

TABLE 2. FRACTIONATION OF PH 5.4, 0.25M $(NH_4)_2SO_4$—SOLUBLE PITUITARY EXTRACTIVES WITH $(NH_4)_2SO_4$ AT PH 6.5 TO 7.0.

Concentration of $(NH_4)_2SO_4$	Globulins precipitated	Growth hormone	Water-soluble proteins	LH	Thyrotropin	FSH
mols/L	*gm./kg.*[1]	R.U./kg.	*gm./kg.*[1]	R.U./kg.	C.U./kg.	R.U./kg.
1.4	6.13	25,000	0.51	2000	None	None
1.6	1.66	64,000	0.31	4000	None	None
1.8	1.21	Trace	0.63	4000	300	None
2.0	0.64	None	0.61	4000	350	None
2.2	0.34	None	0.49	1000	1500	None
2.4	0.32	None	0.66	500	Trace	Trace
2.6	0.28	None	0.81	Trace	None	1000
2.8	0.40	None	0.75	None	None	2000
3.8	1.88	None	2.33	None	None	None

[1] Dry weight of aliquots after dialysis. Assay units are given per kg. of fresh gland.

solution. The FSH fraction contains none of the other pituitary hormones that have been tested, the separation being accomplished from the original extract in one precipitation with no loss of potency. This first precipitate usually contained approximately 2 R.U. per mg.

The further purification of FSH is readily accomplished by redissolving the water-soluble precipitate, after removal of traces of globulins, in 100 cc. of water per kg. of gland. The proteins insoluble in the smaller volume of 2.4M $(NH_4)_2SO_4$ are precipitated and removed in the usual manner. The fraction soluble at 2.4M, but precipitated at 2.7M $(NH_4)_2SO_4$, contains the active material. By repeating this procedure with smaller volumes, preparations have been obtained with little or no loss of total activity which assayed approximately 20 R.U. per mg.

Average results, both with respect to the proteins precipitated and the biological assays of the precipitates at each $(NH_4)_2SO_4$ concentration, are presented in table 2.

CONCLUSIONS AND SUMMARY

A simple procedure is described for extracting the protein hormones of the anterior pituitary gland. By this procedure five of the anterior pituitary hormones have been separated into five different protein fractions.

Only reagents and conditions which are known to have little, if any, deleterious effect on most proteins have been employed. The pH has been retained between 5.4 and 8.0. Only two ions, ammonium and sulfate, in cold aqueous solution, have been added throughout the process.

Of approximately 200 gm. of solids per kg. of fresh, frozen gland, from 58 to 83 gm. were extracted by ammonium hydroxide at pH 8.0. The largest part of these extractives was precipitable near pH 5.4. This precipitate contained the adrenotropic hormone.

Precipitation at pH 5.4 has been carried out in the presence of 0.25 molar ammonium sulfate. As a result the growth and lactogenic hormones as well as thyrotropin and the gonadotropins remained in solution. The growth hormone, as has previously been suggested, is euglobulin in nature and has been concentrated both by dialysis and by precipitation with ammonium sulfate. No loss of original growth activity has been encountered in our preparations and a 160-fold concentration of the hormone has been obtained.

Thyrotropin and the gonadotropins are water-soluble at neutral reactions. Upon the careful, slow addition of ammonium sulfate through a rotating cellophane membrane to their solutions, the luteinizing hormone separates first, then thyrotropin and finally the follicle stimulating hormone. The luteinizing hormone is largely precipitated from the original extract at 2M, the thyrotropic hormone at 2.4M and the follicle stimulating hormone at 2.8M ammonium sulfate.

Upon resolution and reprecipitation at smaller volumes than those of the first extract these respective hormones have been prepared with the following activities per mg. of solids: growth hormone 115 R.U.; FSH, 20 R.U.; LH, 30 to 40 R.U. Per gram of fresh gland: growth hormone, 51 R.U.; FSH, 4 R.U.; LH, 20 R.U.

Half-saturated ammonium sulfate in the cold has been used for the preservation of these protein fractions for further study, and of other proteins not yet assayed for their physiological properties. Further purification of these protein hormone fractions and their characterization as chemical individuals will be reported later.

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THE COMPARATIVE ASSAY OF GONADOTROPIC SUBSTANCES ON RATS, MICE AND CHICKS[1,2]

JOHN S. EVANS, LEONARD HINES, ROGER VARNEY AND F. C. KOCH

From the Department of Biochemistry, The University of Chicago
CHICAGO, ILLINOIS

ONE OF THE MAJOR PROBLEMS in the fractionation of gonadotropic substances is the development of suitable assay methods. The literature contains numerous methods developed by different workers but one is unable to convert the proposed units of one worker into the units of another because of the different methods used and the different types of preparations studied. The purpose of this study is to compare the results obtained from the assay of gonadotropic preparations from sheep pituitary glands, normal male urine, menopause urine and pregnant mare serum on the immature female rat, the immature female mouse, and the 1-day-old chick.

MATERIALS AND METHODS

The anterior pituitary preparation used in this study was prepared by suspending the powdered, acetone-desiccated glands in 0.25% ammonium hydroxide for 24 hours. The PH of the extracting medium was then adjusted to 5.0 to 5.6, and the gland residue centrifuged off. The gland residue was re-extracted with an acetic acid buffer (PH 5.6). The combined extracts were mixed with 3 volumes of 95% ethyl alcohol and placed in the freezer (−3° C) for 24 hours. The resulting precipitate was centrifuged off and dried with either absolute alcohol or acetone and then ether. The dried precipitate is referred to as "pituitary powder A" in this paper.

Gonadogen, a preparation from pregnant mare serum, was supplied by Dr. George Cartland of The Upjohn Company. The assays on this material are expressed in terms of the manufacturer's units. Cartland and Nelson (1) define their unit "as the minimum total dose of hormone which, administered to 21- to 23-day-old rats weighing 35 to 45 gm. in 3 equal subcutaneous injections at daily intervals, will produce at autopsy, 96 hours after the first injection, a mean ovarian weight of 65 mg. which is 4 to 5 times that of the controls."

Prospermin, prepared from normal male urine, and gamone, from menopause urine, were supplied by Dr. J. A. Morrell, E. R. Squibb & Sons Company. The units used in the assays on these substances are reported in terms of the manufacturer's units. Morrell (2) defines a Squibb unit as the total amount of material which, when injected into a group of 29- to 30-day-old immature female rats for 3 days will cause a uterine response in 50% of the animals. A standard powder is run in parallel. Morrell states that a Squibb unit is equivalent to 5 Levin-Tyndale mouse units.

The rat assays reported in this paper were run by the method of Wallen-Law-

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[2] We acknowledge our thanks to Dr. Edwin F. Pike of Armour & Company for the fresh pituitary glands, Dr. George F. Cartland of the Upjohn Company for the gonadogen, and Dr. J. A. Morrell of Squibb & Sons for the prospermin and gamone.

rence and Van Dyke (3). Twenty-one-day-old female rats were injected subcutaneously once daily for 4 days and, 48 hours after the final injection, the ovaries and uteri were freed from any adhering mesentery and weighed on a Roller-Smith torsion balance.

The mouse assays were conducted by a method similar to that used by Hamburger and Pedersen-Bjergaard (4). Female mice, weighing 6 to 8 gm. were injected subcutaneously twice daily for 3 days, and 48 hours after the final injection, the ovaries and uteri were freed from any adhering tissues and weighed on the torsion balance.

The chick assay was developed in this laboratory and was conducted as follows.

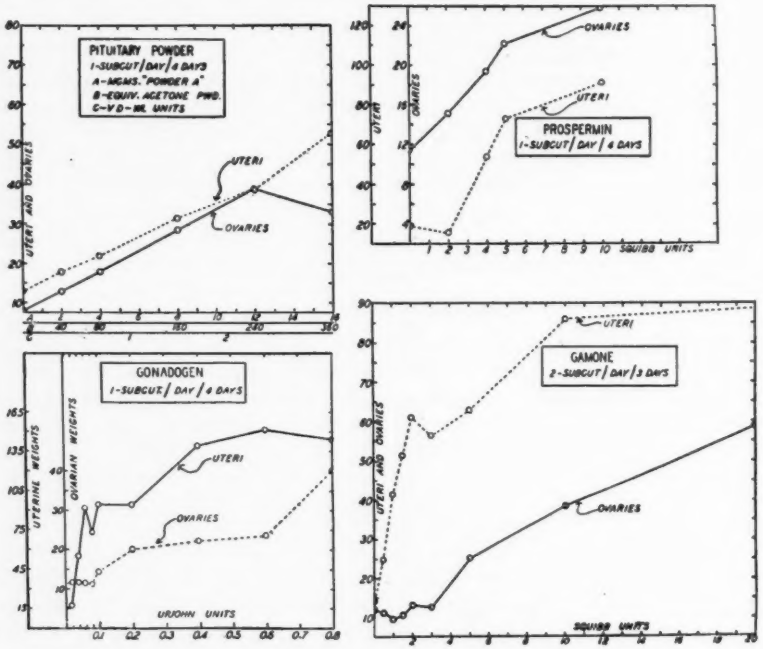

Fig. 1. Gonadotropic assays on the rat with glandular, urinary and blood serum preparations. Animals killed 48 hours after last injection.

One-day old cockerels were divided into groups of 20 each. Each group was injected twice daily for 5 days. Injections were made subcutaneously in the front of the legs, alternating the sides at each injection. Care was taken to use a small needle to avoid leakage. Eighteen hours after the final injection, the testes and the thyroids were weighed on the torsion balance.

EXPERIMENTAL RESULTS

The results of the rat assays are given in figure 1. The ovaries of the rats receiving the pituitary preparations were filled with corpora lutea. It is to be noted that the rat uterus is not a more sensitive indicator than the ovary to the glandular preparation. The uteri were mostly thick-walled with a few of the thin-walled estrous type. The preparation used was unfractionated and the relative amounts of FSH and LH no

doubt play an important part in the amount of the mixture required to produce a uterine response. Pregnant mare serum, normal male urine, and menopause urine preparations which have been shown to be follicle-stimulating substances, produced follicular stimulation at low levels and luteinization at the higher levels. In the case of the last three products, the rat uteri are much more sensitive indicators than the ovary. It should be noted that per Squibb unit, gamone at low levels is very much more active as measured by uterine weight than prospermin. At higher levels the differences are not so striking.

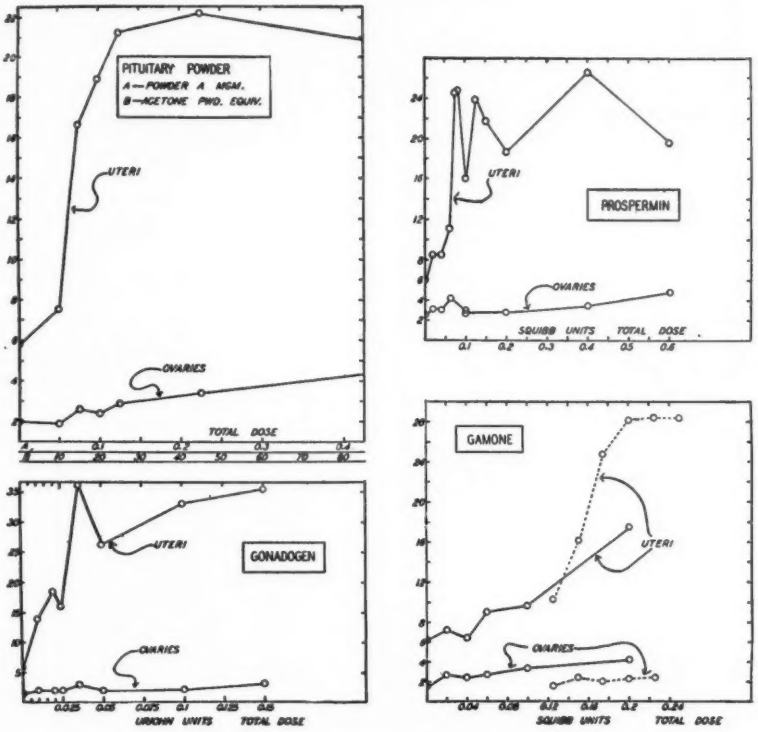

Fig. 2. GONADOTROPIC ASSAYS ON THE MOUSE. Two subcutaneous injections per day for 3 days, organs weighed 48 hours later.

The results of the mouse assays are shown in figure 2. In the mouse, the uterus is a very much more sensitive indicator than the ovary to all the preparations with the possible exception of castrate or menopause urine (gamone).

The results of the chick assays are shown in figure 3. The testes of the chick respond to the injection of pituitary extracts, pituitary suspensions, and pregnant mare serum, but not to pregnancy urine, menopause urine (gamone), and normal male urine (prospermin) preparations. The thyrotropic activity of the pituitary extracts can be assayed at the same time. The pregnant mare serum preparation showed no thyrotropic activity. The left gonad of the 1-day-old Leghorn pullet was found to be less sensitive to pituitary extracts than the testes of the 1-day-old Leghorn cockerel.

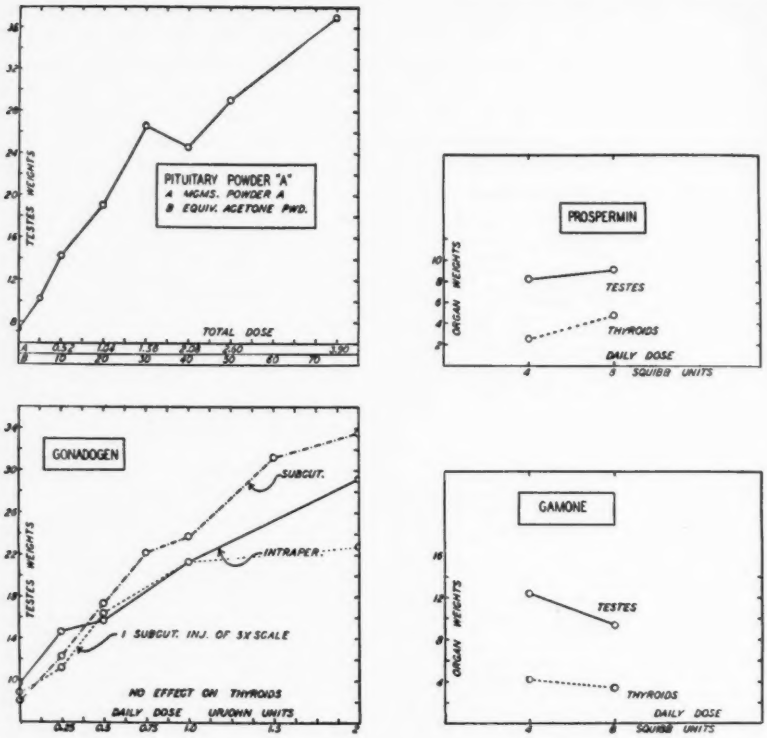

Fig. 3. GONADOTROPIC ASSAYS ON THE CHICK. Two subcutaneous injections per day for 5 days, organs weighed on the 6th day.

DISCUSSION

A comparative summary of our results by the various assay methods is given in table 1 and the relative amounts of material required to produce 100% increases in

TABLE 1. A COMPARATIVE SUMMARY OF THE RESULTS FROM GONADOTROPIC ASSAYS

	Species and organs	Pituitary powder *A*	Mare serum, gonadogen	Male urine, prospermin	Menopause urine, gamone
		mg.	*Upjohn* U.	*Squibb* U.	*Squibb* U.
Minimum doses to produce 100% increases in organ weights	Rat uterus	5.3	0.03	3.5	0.75
	Rat ovary	4	0.6	6.0	4.25
	Mouse uterus	0.06	0.01	0.065	0.13
	Mouse ovary	0.35	0.15	0.6	0.1
	Chick testis	0.65	0.9	8	toxic
Organ and species ratios for the minimum doses	Rat O/Rat U	0.75	20	1.7	5.6
	Mouse O/Mouse U	5.9	Ca 15	9.2	0.8
	Rat O/Mouse O	11	4	10	42
	Rat U/Mouse U	88	3	50	6
	Chick T/Mouse U	10	90	120	—

TABLE 2. RELATIVE AMOUNT OF GONADOTROPIC SUBSTANCE REQUIRED BY EACH SPECIES FOR THE 100% INCREASE IN ORGAN WEIGHTS

Gonadotropin preparation	Rat ovary	Rat uterus	Chick testis	Mouse uterus
Pituitary powder A	66	88	11	1
Pregnant mare serum, gonadogen	60	3	90	1
Male urine, prospermin	92	54	123?	1
Menopause urine, gamone	33	5.8	toxic	1

The absolute amounts required will depend upon the assay method used. The values given are for the particular methods described.

organ weights are given in table 2. The amount of material required to produce a 100% increase in the weight of the mouse uterus is taken as unity.

Examination of figures 1, 2 and 3 and table 1 reveals that the 4 gonadotropins act very differently quantitatively in the 3 species and even in the same species depending on the organ weight used as a measure. Even the 2 urine preparations act very differently on the Squibb unit basis. To illustrate more specifically, it requires approximately 4 times as many Squibb units of prospermin as of gamone to produce the 100% increase in the rat uterus and only 1.4 times the amount for the same change in the rat ovary. In the mouse the orders are reversed. Here only one-half the number of Squibb units of prospermin as of gamone are required for the 100% increase in the mouse uterus, but 6 times the amount for the same result in the mouse ovary. In the chick the testis response requires very high doses of prospermin and the gamone is too toxic to use. The results with pituitary powder A and gonadogen are equally irregular. Thus, the ratio of mg. of powder A to Upjohn units of gonadogen to produce the 100% weight increase of the rat uterus is 176, but for the rat ovary it is 6.6, for the mouse uterus, 6, for the mouse ovary, 2, and for the chick testis, 0.7. Comparison of the ratio of mg. of powder A to Squibb units of prospermin comes the nearest to a consistent agreement in the different species. Thus, for rat uterus it is 1.5, for rat ovary, 0.66, for mouse uterus, 1.0, for mouse ovary, 0.6, and for chick testis, 0.08. The only consistent finding obvious from tables 1 and 2 is the high sensitivity of the immature mouse uterus to the 4 types of the gonadotropins investigated. Inspection of table 2 reveals a rather close agreement in the relative amounts of gonadogen and gamone required for the mouse and rat uterine responses and a much higher ratio for the relative amounts of powder A and prospermin.

These different responses cannot be explained satisfactorily at the present time. The main difficulty is that we are using impure gonadotropin preparations. All of them may contain true follicle stimulating substances, some luteinizing material, interstitial cell-stimulating material, augmenting as well as inhibiting factors and other inert or toxic substances in different proportions. Whether the true follicle-stimulating substances or other gonadotropic factors are identical in any of the preparations, and to what extent we have varying amounts of luteinizing material present in the preparations, or how much we are complicating the assay methods by not eliminating the test animal's own pituitary secretions by using hypophysectomized animals remains to be determined.

A direct comparison of our results with those of others is more difficult because slight differences in the details of the assay methods, different methods of preparation, differences in the age and origin of the test animals, and other variables complicate the problem still more. Brief consideration of the main contributions by others to this comparative aspect of the pituitary problem, however, shows close agreement with our general conclusions on the relative merits of the different methods of assays.

Rat Ovarian and Uterine Responses

It is now generally agreed that the rat uterine response to gonadotropins is much more sensitive than the rat ovarian response. The former is primarily an FSH response, the latter may represent an FSH plus LH response. Heller et al. (5, 6) found that the rat uterus is 8 times as sensitive as the ovary toward crude suspensions of castrate rat pituitaries. They also reported a greater sensitivity toward urinary preparations. Cartland and Nelson (1) with pregnant mare serum preparations reported a 10- to 15-fold sensitivity for the rat uterine response, but considered the ovarian weight increase a better quantitative response. They state that the uterine response is a very sensitive qualitative test and that it represents primarily the FSH factor. With higher doses of their material they always observed a greater ovarian response and luteinization. D'Amour and D'Amour (7) from a comparative study on the assay of gonadotropins from pregnancy urine, placenta and sheep pituitaries concluded that the immature male rat is more sensitive to the first two, that corpus luteum formation was a matter of high enough dosage, that the opening of the vagina was a very unreliable criterion, that the positive estrous vaginal smear was very sensitive for urinary preparations, and that ovarian weight was the "most objective" criterion although at times a marked ovarian response resulting from glandular preparations gave negative estrous smears. Unfortunately, the authors did not weigh the uteri. Cohen and Ardoint (8) also agree that the rat uterine response is a very sensitive indicator.

However, Lauson et al. (9) claim that a greater rat uterine response to pituitary FSH is obtained if the LH factor is added also. Although they claim that a uterine response cannot be considered a reliable measure of FSH concentration, they, nevertheless, recommend the uterine response as a very sensitive test. No doubt the difficulty of separating the FSH and LH factors satisfactorily for assay purposes and the elimination of the factor or factors introduced by the test animal's own pituitary are the main difficulties involved in routine assays. The ideal method is to use only hypophysectomized animals for assay purposes.

The Mouse Assay

Levin and Tyndale (10) studied the application of the mouse uterus to the assay of gonadotropin preparations from castrate urine, normal male urine, pregnancy urine, pregnant mare serum and FSH from sheep pituitaries and concluded that male and castrate urine preparations give essentially the same quantitative response, that the glandular preparation gives a flatter curve, and that the method is not as accurate for the chorionic substances, pregnancy urine extracts, and pregnant mare serum, as for castrate urine. Hamburger and Pedersen-Bjergaard (4) studied the mouse and rat assay of pregnancy urine and pregnant mare serum. They concluded that the ovarian weight response is the best method for standardizing the pregnant mare serum and that the rat gives the most consistent results, but that the vaginal smear of immature rats or the determination of corpora lutea in the ovaries of mice are the best suited for the assay of the gonadotropic agent from pregnancy urine. Rowe and coworkers (11) claimed that the use of immature mice is less practical than the use of rats and no more accurate than other methods of assay. They found that four of their rat units (based upon the production of corpora lutea in rats 26 days old at the start of injection) are equivalent to one Katzman-Doisy (12) unit (mouse-vaginal smear). In a similar manner, they found that the rabbit ovulation unit of Friedman (13) is equivalent to 1 R.U. per kg. of body weight of rabbit.

The Chick Assay

Numerous workers have shown that the testes of the dove or immature pigeon will respond to the injection of pituitary extracts or to homeotransplants. Riddle and Polhemus (14) showed that the testis of the dove is not responsive to the injection of pregnancy urine preparations. Evans and Simpson (15) showed that the dove testis reacts to the injections of pregnant mare serum, but that it requires a higher number of rat units of this material than of their pituitary extracts to produce the same effect. Hamburger (16) observed that the injection of pregnant mare serum into young cockerels produced a rapid growth of the testes and comb of the injected animals. Byerly and Burrows (17) studied the response of the 1-day-old chick to pregnant mare serum. They found a straight line relationship when the logarithm of the testes weight was plotted against the logarithm of the dosage. They proposed the following chick unit: "a male chick unit of gonadotropic hormone be defined as that amount of material which when injected subcutaneously into a male chick during the first day after hatching will produce testes double the weight of control testes 72 hours after the time of injection." These authors found that about 10 R.U. (Cole and Saunders units) were required to double the weight of control testes when injections were made in the manner described.

SUMMARY

In the assay of unfractionated pituitary extracts, the mouse uterus is about 66 times as sensitive as the rat ovary, and about 10 times as sensitive as the chick testes. In the assay of pregnant mare serum preparation (gonadogen), the mouse uterus is about 60 times as sensitive as the rat ovary, and about 90 times as sensitive as the chick testes. In the assay of normal male urine preparation (prospermin), the mouse uterus is about 90 times as sensitive as the rat ovary, and about 55 times as sensitive as the rat uterus. The response of the chick testes to normal male urine is doubtful. The mouse uterus is about 30 times as sensitive as the rat ovary to menopause urine preparation (gamone) and about 6 times as sensitive as the rat uterus. The response of the chick testes to menopause urine is doubtful.

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COMPARATIVE STUDY ON THE AUGMENTATION OF THE ACTION OF GONADOTROPINS FROM VARIOUS SOURCES IN THE RAT, MOUSE AND CHICK[1,2]

JOHN S. EVANS, LEONARD R. HINES, JOSEPH J. CEITHAML AND F. C. KOCH

From the Department of Biochemistry, The University of Chicago
CHICAGO, ILLINOIS

MANY OBSERVATIONS (1, 2, 3) have been made which clearly show that the response of the immature female rat to gonadotropic material of glandular origin is complicated by augmenting effects as well as by antagonists. The purpose of this study is to investigate further the degree of augmentation brought about by various reagents with 4 types of gonadotropin preparations in 3 species and to attempt to throw more light on the mechanisms involved.

Assay methods. The rat method is similar to that used by Wallen-Lawrence (4). Female rats, 21 to 23 days of age, were injected subcutaneously once daily for 4 days and autopsied 48 hours after the final injection. The ovaries and uteri were dissected free from adhering tissues and fat. The uteri were trimmed by cutting through the utero-tubal junction and through the junction of the cervix and the uterus, and freed from fluid by pressing against paper toweling. The organs were weighed on a Roller-Smith torsion balance.

The mouse assays were conducted by the method of Hamburger. Immature female mice, weighing 6 to 8 gm., were injected twice daily for 3 days, and 48 hours after the last injection the ovaries and uteri were dissected and weighed.

The chick assays were conducted by injecting groups of about 20 1-day-old cockerels subcutaneously in the front of each leg twice daily for 5 days and weighing the testes 18 hours after the last injection. In certain of the experiments, the injection period was shortened to 2 days.

Gonadotropin preparations. The glandular product, prep. 137, was prepared by suspending acetone-dehydrated sheep pituitaries in 0.25% ammonium hydroxide for 24 hours, adjusting the PH to 5.0 with acetic acid and centrifuging off the gland residue. The active material was precipitated from the supernatant solution by adding 3 volumes of ethyl alcohol. The precipitate was centrifuged off and extracted with 0.25% acetic acid. This solution is called prep. 137. The pregnancy urine preparation was made by adsorbing the gonadotropic substance on benzoic acid and subsequently precipitating the active material by the addition of a large excess of acetone. Gonadogen is the Upjohn Company's preparation made from pregnant mare serum. Prospermin and gamone are the Squibb & Sons' preparations made from normal male and menopause urines respectively.

Effect of copper and zinc salts in the rat. The results in table 1 show that the addition of zinc or copper salts to sheep pituitary extracts produced an augmentation in

[1] These investigations were supported by a grant from Armour & Company.

[2] We are indebted to Dr. George F. Cartland of the Upjohn Company for the gonadogen, to Dr. J. A. Morrell of Squibb & Sons for the prospermin and gamone, to Dr. Edwin F. Pike of Armour & Company for the pituitary glands, and to Dr. G. H. A. Clowes of Eli Lilly Laboratories for the protamine used in these studies.

the response of the rat ovary, but when the extract and Cu or Zn salts were injected separately little or no augmentation was observed. The slight increase obtained when the Cu salts and the extract were injected separately may have been due to the slight mixing of the two solutions in the subcutaneous area after the injection. The addition of Cu and Zn salts to the chorionic substances, pregnancy urine and pregnant mare serum preparations, did not produce augmentation. The slight increase in the uterine weight may reflect a slight effect but since the uterine response is already well up on the uterine curve, it probably does not have any meaning. Addition of Zn salts to solutions of prospermin or gamone did not produce an augmentation. Hemin augmented the response of the rat ovary to a given dose of pituitary extract.

TABLE 1. THE EFFECT OF COPPER SALTS, ZINC SALTS, AND HEMIN ON THE ACTION OF VARIOUS GONADOTROPINS IN THE RAT

Gonadotropic substance	Injected together		Injected separately	
	Ovarian wt.	Uterine wt.	Ovarian wt.	Uterine wt.
	mg.	mg.	mg.	mg.
Prep. 137, 0.5 cc.	20.9	32.7		
Prep. 137, 0.5 cc.+1.6 mg. $ZnCl_2$	62.7	57.4	26.7	28.7
Prep. 137, 0.5 cc.+2.7 mg. $CuSO_4$	86.7	68.8	33.2	29.6
PU[1] Prep. 99, 1.5 mg.	17.7	78.0		
PU[1] Prep. 99, 1.5 mg.+2.7 mg. $CuSO_4$	24.2	97.9	14.3	65.2
Gonadogen, 0.5 R.U.[2]	22.5	123.6		
Gonadogen, 0.5 R.U.[2]+2.7 mg. $CuSO_4$	18.3	148.0	27.2	107.9
Prospermin, 5 Sq. R.U.[2]	22.3	115.2		
Prospermin, 5 Sq. R.U.[2]+2.1 mg. ZnAc.	28.3	110.2		
Gamone, 5 Sq. R.U.[2]	23.6	111.5		
Gamone, 5 Sq. R.U.[2]+2.1 mg. ZnAc	23.6	83.4		
Prep. 137, 1 cc.	27.8	26.9		
Prep. 137, 1 cc.+5 mg. hemin	36.2	40.3		

[1] PU refers to prenancy urine preparation.
[2] R.U.=rat units as expressed by the manufacturer.

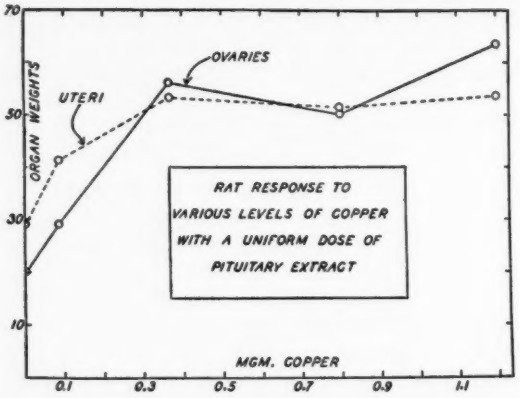
The results of our experiments (table 2) and those of Pfeiffer (16) indicate that the action of augmenting agents, such as Cu or Zn salts, is not due in any way to its action upon the animal's endogenous hormones.

TABLE 2. EFFECT OF COPPER AND ZINC SALTS UPON THE ENDOGENOUS HORMONES OF THE IMMATURE RAT

Injected material	Ovarian wt.	Uterine wt.
	mg.	mg.
0.9% NaCl	11.5	19.0
0.012 N $CuSO_4$	10.5	18.9
0.012 N $ZnCl_2$	10.1	16.6

The effect of increasing the concentration of Cu per hormone unit. Saunders and Cole (5) showed that the addition of varying amounts of zinc sulfate or of casein to a constant dose of pituitary extract produced increased ovarian weights in the rat with increased dosage. Their results were confirmed in our laboratory by adding various amounts of copper sulfate to a constant dose of extract (fig. 1). Ovarian weights increased with increasing amounts of salt added until a total dose of 1 mg. of $CuSO_4$ was reached. At this level, the response tended to plateau off. At about the same level, the toxic effects of the Cu salts are first noticeable by a loss of weight, loss of hair and the development of sores at the site of injection on the animals.

Effect of protamine in the rat. Fevold, Hisaw, Hellbaum and Hertz (2) precipitated their active material in the form of tannates and injected suspensions of their precipitates. They believed that the tannic acid formed a combination which retarded the rate of absorption of the hormone. McShan and Meyer (6) have pointed out that there is not always a correlation between the formation of insoluble precipitates and



the production of augmentation. Bates and Riddle (7) found that prolactin was precipitated from solution by the addition of Cu and that the supernatant solution was very potent with respect to gonadotropic and thyrotropic hormones. Their findings suggest that the augmentative power of Cu salts does not lie in the formation of an insoluble precipitate. Table 3 shows the results of an experiment in which a total dose of 2 mg. of protamine (Lilly) was combined with a total dose of 0.5 cc. of pituitary extract at various pH levels. When the solution was pH 6.5 or above, a flocculent precipitate occurred, but no augmentation was produced. No augmentation was produced at lower pH levels (table 3).

TABLE 3. EFFECT OF THE ADDITION OF PROTAMINE TO PITUITARY EXTRACTS IN THE IMMATURE RAT

No.	Total dose pituitary 137	Total dose protamine	How administered	pH	Ovarian wt.	Uterine wt:
	cc.	mg.			mg.	mg.
1	0.5	0.0	—	4.5	32.2	45.1
2	0.5	2.0	together	4.5	36.9	48.2
3	0.0	2.0	—	—	9.7	17.2
4	0.5	2.0	separately	4.5	32.3	45.8
5	0.5	0.0	—	4.5	31.0	33.1
6	0.5	2.0	together	6.8	20.9	32.3
7	0.5	2.0	together	10.8	25.8	48.9

When the solutions were injected separately they were injected subcutaneously at different sites.

Breneman (8) showed that the addition of a suspension of 0.25 mg. hemin to pituitary extract did not produce any augmentation. If sufficient pyridine was added to dissolve the hemin, augmentation did occur. McShan and Meyer (6) have suggested that this may be due to the formation of a hemochromogen-like compound. It is apparent that retarding of absorption by the formation of insoluble precipitates is probably not the only mode of action of an augmenting agent.

Effect of adrenalin on the gonadotropic response in the rat. Tannic acid (2), zinc

salts (1), merthiolate (9), casein (5), and other compounds have been reported to act by slowing down the rate of absorption and possibly reducing the toxicity. Adrenalin, by inducing local vasoconstriction and thus retarding the local absorption, prolongs the effects and reduces the toxicity of cocaine and similar local anesthetics. Hence adrenalin was added to pituitary extracts and gonadogen in order to retard the rates of absorption by local vasoconstriction. The effects of adrenalin upon these substances are shown in table 4.

TABLE 4. THE EFFECT ON THE RAT ASSAY OF THE ADDITION OF ADRENALIN TO GONADOTROPIC SUBSTANCE

No.	Gonadotropic substance	No. of daily injections	Addition	Ovarian wt.	Uterine wt.
				mg.	mg.
1	0.5 cc. pituitary 137	2	—	33.8	42.7
2	0.5 cc. pituitary 137	2	Adrenalin[1]	75.0	65.0
3	0.5 cc. pituitary 137	2	—	28.9	42.0
4	0.5 cc. pituitary 137	2	Adrenalin	26.9	35.9
5	1.0 cc. pituitary 137	2	—	62.9	48.0
6	1.0 cc. pituitary 137	2	Adrenalin	100.0	84.3
7	0.83 R.U. gonadogen	1	—	35.8	167.1
8	0.83 R.U. gonadogen	1	Adrenalin	30.6	149.1

[1] In 2, 6 and 8, the mixture of gonadotropin and adrenalin was injected subcutaneously. In 4, the adrenalin and pituitary extract 137 were injected separately. The concentration of adrenalin in 2, 6, and 8 was 1:10,000; in 4, an equivalent amount of 1:10,000 adrenalin was injected.

The addition of adrenalin to the extracts from sheep pituitary produces an augmentation in the ovarian and uterine responses in the rat. When the adrenalin and the extracts were injected separately, no augmentation was observed. No augmentation was observed when adrenalin was added to the pregnant mare serum preparation. Possibly this negative effect is due to the fact that normally the hormone is very slowly destroyed and excreted from the body.

Administration of the hormone by heart puncture. In order to test the hypothesis that the augmentation, produced by the addition of $CuSO_4$ and other substances is due to a delay in the rate of absorption, two aliquots of the pituitary extract, one with and the other without $CuSO_4$, were each injected into rats subcutaneously

TABLE 5. ADMINISTRATION OF THE HORMONE BY HEART PUNCTURE IN THE IMMATURE RAT

Substances injected	Mode of injection	Ovarian wt.	Uterine wt.
		mg.	mg.
Pituitary 137+$CuSO_4$	Subcutaneous	36.5	65.3
Pituitary 137+$CuSO_4$	Heart puncture	15.5	22.9
Pituitary 137	Subcutaneous	15.2	21.8
Pituitary 137	Heart puncture	14.8	22.2

and directly into the heart. The results are shown in table 5. The preparation containing $CuSO_4$ when injected by heart puncture gave approximately the same results as when the solution without the $CuSO_4$ was injected either subcutaneously or by heart puncture. The same total dose with the addition of $CuSO_4$ when injected subcutaneously produced marked augmentation.

Effect of vasodilators and diuretics in the rat. Our results thus far indicate that the augmenting agents act by retarding the rate of absorption. The converse of the action of adrenalin was tried in order to see if a vasodilator would increase the rate of ab-

TABLE 6. EFFECT OF SODIUM NITRITE AND DIURETICS ON THE RAT RESPONSE TO PITUITARY EXTRACT

Total dose prep. 137	Addition	Ovarian wt.	Uterine wt.	Remarks
cc.		mg.	mg	
1.5	—	28.5	41.3	1 injection per day
1.5	0.6 mg. $NaNO_2$	31.0	32.6	1 injection per day
1.5	1.67 mg. theophyllin	26.7	56.0	1 injection per day
1.5	1.5 mg. theobromine sodium acetate	32.2	47.6	1 injection per day

sorption. The first vasodilatin used was sodium nitrite (table 6). This substance has only a very temporary vasodilating action so the negative results are not unexpected. Isomannide dinitrate[2] which has been shown to have a more prolonged vasodilating action in the dog (10) was used in the later experiments. The action of the isomannide dinitrate produced an increase in the response to a given dose of hormone rather than the expected decrease. This may be due to a general vasodilation producing a fall in blood pressure and consequent retention of the hormone in the blood stream (see table 7).

TABLE 7. EFFECT OF ISOMANNIDE DINITRATE UPON THE RAT RESPONSE TO PITUITARY EXTRACT

Total dose prep. 137	Total dose[1] vasodilator	Ovarian wt.	Uterine wt.	Remarks
cc.	cc.	mg.	mg.	
1.0	—	44.9	49.3	Injected subcutaneously
1.0	0.015	42.9	53.1	Injected subcutaneously
1.0	0.03	68.1	51.6	Injected subcutaneously
1.0	0.06	61.9	57.5	Injected subcutaneously
1.0	0.16	54.4	56.5	Injected subcutaneously
1.0	0.32	48.0	49.8	Injected subcutaneously
1.0	2.0	55.2	46.0	Isomannide made fresh daily, injected subcutaneously
1.0	8.0	54.8	56.2	Isomannide made fresh daily, given orally, stomach tube.

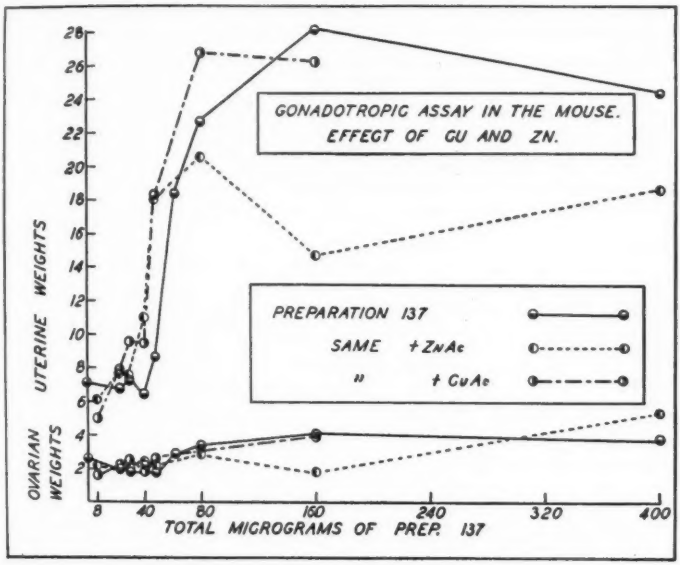
[1] The isomannide dinitrate was given as a 1:1000 solution. The solutions were injected twice daily for a period of 4 days and the animals autopsied about 40 hours after the final injection.

The effect of the addition of diuretics such as theophyllin and theobromine to the extract was studied (table 6). Theophyllin or theobromine had no effect on the assay. However, the diuretic actions of theophyllin and theobromine are not very marked in the rat (11). This may account for the failure to remove the hormone more rapidly from the circulation of the rat through the kidneys.

Attempts to augment the gonadotropin assay in the mouse. L. C. Maxwell (1) claims that the addition of zinc sulfate to minimal dosages of pituitary extracts exerts a strong estrogenic action on the rat uterus, but often without detectable morphological changes in the ovaries. Hamburger (12) and also Levin (13) have shown that the mouse uterus is very sensitive to substances which stimulate the ovaries to secrete estrogens, hence we were interested to study the effect of Zn, Cu, and adrenalin on the gonadotropic response in the immature mouse.

Figure 2 shows the effect of the addition of Zn and Cu salts upon the mouse assay of preparation 137. At the higher levels the Cu salts caused death. There is a slight

[2] We wish to acknowledge our appreciation to Professor E. M. K. Geiling for this suggestion and to Dr. J. C. Krantz for the preparation used.



shift of the uterine weight curves toward the left but no effect on the ovarian weight. No gross structural changes were observed in the ovaries of the mice. The negative effect of the addition of adrenalin to pituitary extracts upon the mouse assay is shown in table 8.

TABLE 8. EFFECT OF ADRENALIN ON THE GONADOTROPIC RESPONSE IN THE MOUSE

Gonadotropic substance	Body wt.	Ovarian wt.	Uterine wt.	No. of animals
	gm.	mg.	mg.	
0.1 cc. pituitary 137	9	2.3	16.2	7
0.1 cc. pituitary 137 adrenalin, 1:10,000	10	2.6	16.1	5
Controls	7	2.5	7.1	5

Levin and Tyndale (13) have shown that the mouse ovarian weight is a very poor gonadotropic criterion and even with the use of large numbers of animals errors of less than 100% are not avoidable. They also state that castrate urine extracts are less likely to produce luteinization in the mouse than in the rat. Engle (14), however, claims that with pituitary extracts, rats tend to show less luteinization than mice.

Attempts at augmentation in the chick. Breneman (8) reported that the addition of augmenting agents to pituitary extracts produced an increase in the chick testis weight over that expected from a given amount of pituitary extract. The augmentation reported was, however, of a very low order, i.e., chlorophyll produced an increase of 1.6 mg. and auxilin produced an increase of 2.0 mg. in average testis weights. It was felt advisable to repeat the studies with better established augmenting agents.

Our first series of chicks was injected twice daily for 5 days and autopsied about 18 hours after the final injection. The second series was injected twice daily for 2 days and autopsied 18 hours after the final injection. The results are shown in table 9.

Preparations containing Zn salts were made up in such a manner that the concentration of the Zn was 0.012 N. The adrenalin solutions were 1:10,000. Prep. 169

TABLE 9. EFFECT OF AUGMENTING AGENTS ON THE CHICK ASSAY

Total dose	Addition	No. of chicks	Average testes and range	Standard deviation
			mg.	
5-Day Injection Series				
Prep. 169, 10 mg. sheep[1] D.P.	—	20	14.7 7.8–22.7	3.98
Prep. 169, 10 mg. D.P.	ZnAc	8	16.1 6.4–31.2	8.24
Prep. 169, 10 mg. D.P.	Adrenalin	11	14.7 9.1–27.4	5.58
26-S (Beef ext.), 1.0 mg.	—	38	12.7 7.1–24.0	4.64
26-S (Beef ext.), 1.0 mg.	Adrenalin	14	12.1 7.7–28.6	5.47
Controls	—	52	9.9 4.0–21.0	3.80
6.0 mg. PU 92[1]	—	10	11.3 7.9–16.9	3.36
2-Day Injection Series				
Saline controls	—	17	9.9 3.7–16.4	2.72
Prep. 169, 20 mg. sheep D.P.	—	18	15.8 8.3.27.7	5.08
Prep. 169, 20 mg. D.P.	ZnAc	20	19.6 7.3–37.6	7.97
Prep. 169. 20 mg. D.P.	Adrenalin	16	13.3 8.8–26.7	4.21
Prep. 169, 20 mg. D.P.	1.5 mg. P.U. 318	19	19.1 7.7–38.7	7.50
Uninjected controls	—	9	8.2 4.0–18.2	4.42

[1] PU refers to pregnancy urine preparation. 6.0 mg. PU 92 is equivalent to 17.5 cc. urine; 1.5 mg PU 318 is equivalent to 20.0 cc. urine.

Standard derivation $= \sqrt{\frac{\Sigma d^2}{N-1}}$

where d = the difference between the individual testes weights and the average testes weight, N = the number of individuals.

was prepared from sheep pituitary acetone powder and the dosage is expressed in terms of the equivalent of desiccated powder. The beef extract was prepared by extracting desiccated beef powder with dilute acetic acid.

In the 5-day series of injections, the Zn salts produced a slight increase in the average weight of the chick testes. However, the number of birds in this group was small and there was a wide variation in response to the material. In the 2-day series of injections, the addition of either Zn acetate or prolan produced an increase in the

average testes weight of about 3.5 mg. The standard deviation, even with a large series of animals was high. The addition of adrenalin to the extract using either the 2- or 5-day series of injections did not produce any augmentation. Because of the high standard deviation, we are not certain that Zn salts or prolan have any augmentative action in the 1-day-old chick. The wide variation in response to a given dose makes it imperative that one use a large number of animals on a given level.

Since it is possible to measure the thyrotropic activity of a preparation by the chick method it is interesting to note that no augmentation of the thyrotropic hormone was observed when Zn salts or adrenalin were added to the pituitary extracts.

DISCUSSION

The mechanism of the augmentation reaction has been believed to be due to a slower rate of absorption of the hormone with a consequent lowering of its rate of excretion and/or destruction. Tannic acid (2), Zn salts (1), Cu salts (3), merthiolate (9), egg albumin (5), heme (6), yeast extract (3), yeast ash (3), and a group of other substances (15) have been reported as effective augmenting agents. Fevold, Hisaw and Greep (3) claim that the action of Cu salts is not simply due to a delayed rate of absorption, but that Cu acts possibly by 'catalyzing' the synergistic action of the follicle stimulating and luteinizing hormones. They showed that the intravenous administration of copper acetate (10 mg.) produces ovulation in the rabbit. Saunders and Cole (5) confirmed Fevold and coworkers' observation (3) that Cu produces ovulation in the rabbit but that Zn salts, casein, or egg albumin do not do so. Fevold and coworkers (3) claimed that Zn salts augment FSH and FSH plus LH in normal and hypophysectomized rats, whereas Cu salts augment FSH plus LH, but do not enhance FSH alone in hypophysectomized rats. According to them, Cu and Zn salts augment both FSH and FSH plus LH in the normal immature female rat.

Pfeiffer (16) showed that the injection of $CuSO_4$ into constant estrous rats produced only a slight disturbance of the estrous smear. He attributed this to the toxic action of the $CuSO_4$ and concluded that the salt had no effect on the animal's endogenous hormones. He further showed that the injection of Cu salts did not produce excessive luteinization of the ovaries of immature rats. Copper sulfate injected into normal and castrate males bearing ovarian grafts in the anterior chamber of the eye, produced no augmentation on the grafts detectable during the experiment or at autopsy. He pointed out that the negative results obtained may be due to the small amount of the endogenous hormones in the blood stream, to the rapid elimination of the salt or extract, or to the necessity of the formation of a protein-salt complex.

Maxwell (1) showed that the addition of Zn salts to a pituitary extract resulted in an increased response to the extract in the rat, but that by distributing the dosage without the addition of Zn salts, he could obtain nearly the same effect. Maxwell believed that the Zn salts act by delaying the rate of absorption of the extract.

McShan and Meyer (6) showed that whole blood, erythrocytes, hemoglobin and heme augment the response in the rat to an extract of sheep pituitary glands. They attributed the augmentation to the breakdown of these substances into heme. Inorganic iron showed no enhancing activity. The authors point out the possibility of the formation of a hemochromogen-like compound which would be more active than the gonadotropic complex. Krebs (17) showed that heme is a poor 'catalyst' and that when heme is combined with certain nitrogenous compounds to form hemochromogens, the catalytic effect of the heme is dependent upon the group with which the heme is combined. The authors feel that the action cannot be attributed to a delay in the rate of absorption since there was no insoluble precipitate formed

when heme was added to the extract. They pointed out that the formation of insoluble precipitates and augmentation are not necessarily associated. This was also demonstrated in this laboratory when protamine was added to pituitary extracts with the formation of an insoluble precipitate, but augmentation was not produced.

Cassida (18) showed that the augmentative action of beef blood is due to the formed elements rather than to the plasma. Kraatz (19) claims that leucocytes possess an augmenting action. Preliminary experiments by him indicated that there is a rise in eosinophil content after the injection of Zn salts, either alone or in combination with FSH, into immature females, while injections of the hormone alone tend to lower the eosinophil number. Kraatz suggested that the number of circulating eosinophils may be an indication of the luteinizing activity and that these cells may play a rôle in the control and transport of the luteinizing hormone.

In our studies, we have confirmed the work of numerous investigators who showed that Cu salts, Zn salts, and heme augment pituitary extracts in the assay based upon the rat ovary. The general conception is that such substances act by delaying the rate of absorption. That a delay in the rate of absorption will produce an augmentation is shown by our adrenalin and heart puncture experiments. That all gonadotropins do not react in a similar manner is evident from the work on extracts of pregnancy urine, normal male urine, castrate urine and pregnant mare serum. Neither do all species react, at least to the same degree, to pituitary extracts containing augmenting substances. The fact that agents which are very effective in producing an augmentation of pituitary extracts in the immature female rat have little, if any, effect in other species such as the mouse or chick suggests that the mechanism may not be entirely a delay in the rate of absorption. This is further emphasized by the fact that substances capable of delaying the rate of absorption do not augment other gonadotropins than pituitary extracts. The results of our experiment and those of Saunders and Cole (5) show that there is an optimum amount of augmenting agent which should be added to a given dose of extract in order to obtain the best response. Since many substances augment pituitary preparations and other substances or extracts antagonize the response to pituitary preparations, it is evident that such disturbing factors must not be ignored in assaying gonadotropin preparations.

SUMMARY

The subcutaneous injection into immature female rats of mixtures of Zn salts, or adrenalin with pituitary extracts cause a marked augmentation in ovarian and uterine responses. When injected separately, no such augmentation is observed. Preparations from pregnancy urine, pregnant mare serum (gonadogen), normal male urine (prospermin) and menopause urine (gamone) are not augmented by Zn salts, adrenalin, or Cu salts in the immature female rat. Copper and Zn salts do not augment the animal's endogenous hormones. Heart injection experiments gave additional evidence that in the rat the Cu produces its augmentation in part by delaying the rate of absorption of the hormone. Sodium nitrite, theophyllin and theobromine have no effect on the rat ovarian response to pituitary extracts. Isomannide dinitrite augments the rat ovary response to pituitary extracts. Zinc and Cu salts, when added to pituitary extracts, exert a slight augmentation in the mouse uterine response. Zinc and Cu salts, as well as adrenalin, fail to augment the pituitary extract when the weight of the mouse ovary is the criterion of assay. Zinc salts and adrenalin do not augment the chick testis response to pituitary extracts in a 5-day period of assay. Adrenalin in a 2-day test period produces no augmentation.

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PHYSIOLOGICAL SIGNIFICANCE AND MORPHOLOGY OF THE CARMINE CELL IN THE CAT'S ANTERIOR PITUITARY[1]

H. B. FRIEDGOOD AND A. B. DAWSON

From the Department of Physiology in the Harvard Medical School, and the Biological Laboratories, Harvard University

BOSTON AND CAMBRIDGE, MASSACHUSETTS

IN A STUDY of the anterior pituitary of the female rabbit (1, 2) it was found that striking changes occurred in the composition of the acidophil cells after mating. This reaction was demonstrated by means of a method which separated the acidophils into two distinct tinctorial types (3). The glands were fixed in sublimate-formalin followed by an adaptation of Heidenhain's azan modification of Mallory's connective tissue stain. The ordinary or standard acidophil was colored yellow with orange G. Other acidophil cells which become conspicuous during sexual activity were characterized by the coarseness and refractivity of their granules, and took a brilliant red stain due to their special affinity for azo-carmine. The chromophobes were not colored or appeared a light pink, and the basophils stained blue with analin blue.

In estrous rabbits the modified acidophils (carmine cells) are usually small and inconspicuous, but after mating they increase in size and number and become grouped in large, irregularly circumscribed areas. The carmine reaction is strongest from 1 to 3½ hours postcoitum, after which there occurs an obvious degranulation which is completed between 12 and 18 hours postcoitum. A secondary and less striking reaction appeared 20 to 24 hours after mating. A similar degranulation of acidophils following mating has been noted by Severinghaus (4), but apparently the remarkable granulation and tinctorial difference in certain of the acidophils were not demonstrated by the technic he employed.

Well-recognized peculiarities in the sex cycle of the rabbit make it difficult to ascertain with certainty the exact status of the sexual activity of an animal at the time of an experiment. This is especially true of any attempt to estimate quantitatively the intensity of the estrous state. This circumstance suggested the advisability of extending our observations to the cat which, like the rabbit, normally ovulates only after copulation. The cat has a relatively long, well-defined anestrous period and a characteristic spontaneous behavior pattern during estrus, which can be elicited mechanically by stimulation without exposure to a male (5).

In the present study, of which preliminary reports have been published (6, 7), attention is directed primarily to the presence or absence, distribution and relative number of the carmine cells during the periods of anestrus, proestrus, estrus and the 24 hours which succeed mating. Major changes apparently also occur in the percentage of ordinary acidophils, chromophobes and basophils, but these are not so obvious or striking as the carmine reaction.

In order to avoid possible complications, i.e., residual effects on the pituitary of preceding pregnancy and lactation, or recurrent estrus during the breeding season,

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our early observations were based on cats which had been brought into the laboratory during the long autumnal anestrous period. Some were autopsied while still in the anestrous state, others in the first observed estrus, and others at timed intervals after mating or stimulation of the cervix uteri with a glass rod, according to the method of Greulich (5). Other animals of unknown history were brought into the laboratory during the breeding season and usually autopsied immediately. Most of these were either in an anestrous state or pregnant. The uterus and ovaries were sectioned and studied in those cases in which the phase of the reproductive cycle was not obvious.

Breeding cycle. Although the cat has been used rather extensively for laboratory purposes, detailed statements concerning its reproductive cycles are surprisingly meager and widely scattered. One of the characteristics of the normal cycle is a definite anestrous period lasting usually from September to January (8). This period may vary, however, with the reproductive history of the individual and with climatic, nutritional or other environmental conditions.

Proestrus is not readily recognized by an examination of the living animal and the period is ill-defined. The vaginal smear is characterized by a mixture of large, flattened and small epithelial cells but is not easily differentiated from smears during metestrus and after implantation of the ovum (8). The best criteria are found in the ovaries, tubes and uteri. There is usually a selective enlargement of 3 to 7 follicles and an accompanying atresia of the others. The epithelial lining of the tubes and uterus hypertrophies, but mitotic activity does not increase perceptibly until estrus. Inspection of the ovaries by laparotomy seems inadvisable as surgical intervention appears to interfere with the normal sequence of events after mating (9, 10).

The first estrus may occur during the months of January or February and lasts from 2 to 3 days (8), although many observers state that the period is variable and may be much longer (4 to 12 days, 10; 6 days, 11). Isolated cats may have a recurrent estrus at about 14-day intervals.

The gestation period lasts about 60 days, varying from 56 to 63. Lactation is usually maintained for about 60 days, and estrus normally does not occur during this period, although Bourg (13) cites a case of impregnation 6 weeks after a parturition followed by lactation. The anestrous period following pregnancy without lactation lasts from 2 to 4 weeks according to Evans and Swezy (11), and from 3 to 6 weeks according to Longley (12). Stadler (11) has found that if coitus is prevented during the first estrus after pregnancy or lactation, the succeeding estrous cycles are peculiar in that several short recurrences of estrus take place, and it becomes impossible to correlate the follicular and estrous cycles.

Isolated females under our laboratory conditions did not always come into the first estrus in January or February. Some showed first heat in March, others as late as June, and others were never found in estrus although under observation until late in July. The animals were well nourished and kept in inside, roomy, air-conditioned quarters. Furthermore, under laboratory conditions, the recurrent estrus at 14-day intervals was not always the rule.

It is generally conceded that ovulation in the cat is not spontaneous, but is induced by coitus or a comparable stimulus (5). There are several references, however, to the possible occurrence of ovulation without any known stimulation. Moreover, ovulation may be brought about by appropriate treatment with FSH and LH (8, 14).

The observations of Courrier and Gros (15) indicate that ovulation usually occurs between 26 and 27 hours after mating. This is in general agreement with the data of Van der Stricht (16) and Greulich (5). In one case Greulich found tubal ova 28 hours after artificial stimulation. Although there is probably some individual variation in the time which elapses between coitus and the ovulatory response, ovulation appears to take place from 24 to 30 hours after mating. Fertilization usually occurs in the first half of the second day. Ovulation does not invariably follow coitus. In certain cases some follicles rupture, while others become greatly enlarged and form either simple follicular or luteal cysts. In other cases none may respond, and all become either cystic or atretic.

In order to study intelligently the changes occurring in the pituitary in the period be-

tween coitus and ovulation it became necessary to establish criteria of successful mating. The changes in the vaginal smears and uterus do not take place early enough to be of value in this regard (8). However, the changes in the Graafian follicle and ovum occur much earlier, and it is possible to recognize initial changes in the region of the cumulus as early as 6¾ hours postcoitum. There occurs, thereafter, a regular sequence of changes which can be followed up to the establishment of the second polar spindle at 22 to 24 hours (17).

During the first 6 hours after mating the interpretation of the changes occurring in the pituitary must be based largely on its cytological picture alone, without definite confirmation by related progressive changes in the reproductive tract. From a practical viewpoint this has caused little difficulty since the differences between the pituitaries removed from unmated estrous and mated cats are very striking. When these are correlated with the histological picture of the pituitaries removed after 6 hours postcoitum, the early changes are readily interpreted.

The pituitary gland. The major subdivisions of the pituitary gland of the cat are sharply separated histologically into posterior lobe (infundibular process), intermediate lobe and anterior lobe proper. The pars intermedia completely invests the posterior lobe and in turn is clearly separated from the pars anterior by the persistence of a well-defined residual lumen. The pars tuberalis, which is characteristically vesicular in appearance, is a thin sheet of tissue investing the infundibular stalk. However, it is greatly thickened where it joins the median, antero-dorsal margin of the pars anterior and at this point is distinctly separated into a peripheral, loose vesicular and a central, compact zone. The compact tissue is continuous with similar tissue of the anterior lobe, forming an irregular area which occupies the median antero-ventral region of the pars anterior and extends centrally to the residual lumen. This entire median area, which is characterized by a limited number of acidophils and a predominance of basophilic and chromophobic cells, has been designated the zona tuberalis of the pars anterior (18). Thus, in frontal sections, the anterior lobe proper, seen as a thickened crescent whose horns clasp the intermediate and posterior lobes, is divided by this basophilic area into two lateral wing-like halves (fig. 5). The latter are characterized by a limited number or almost complete absence of basophils (fig. 1).

In this study the zona tuberalis of the pars anterior was an important landmark, since the cells reacting specifically with azo-carmine always appear on the periphery of the pars anterior immediately adjacent to the area of the zona tuberalis.

The carmine cell. The cell reacting with azo-carmine in the cat pituitary is similar to, but not identical morphologically with, that previously described in the rabbit. With the fixation used, the granulation is coarser than in the ordinary acidophil, but the large conspicuous negative image of the Golgi apparatus seen in the carmine cells of the rabbit is not so obvious. When the carmine cells are few and scattered, they do not have a typical form and are wedged between the ordinary acidophils (fig. 4). When numerous or predominating in any area, they are found in a characteristic alveolar pattern (fig. 3) which enables them to be recognized even without differential staining.

The cells forming alveolar patterns are pyramidal in shape, with the granules accumulated in the basal pole and the nucleus centrally located or displaced toward the center of the alveolus. The extreme apical ends of these cells are frequently not granulated. It is assumed that in such cells that Golgi apparatus occupies the non-granular region, since a negative image of this structure is not usually seen in the granulated basal region.

OBSERVATIONS

Anestrous cats. The pituitaries of 14 anestrous cats have been studied (table 1). Six cats (1A to 6A) were autopsied during the months of October, 1937, and March, 1939, as soon as they reached the laboratory. Those examined in December, 1937, and January and February, 1938 (7A to 14A) were kept under observation in the laboratory for a number of months, but were never actually observed in estrus.

There was a complete absence of carmine cells in the anterior pituitaries of all these animals. The median area of the zona tuberalis was definitely segregated from the remainder of the gland and was composed largely of compactly arranged baso-

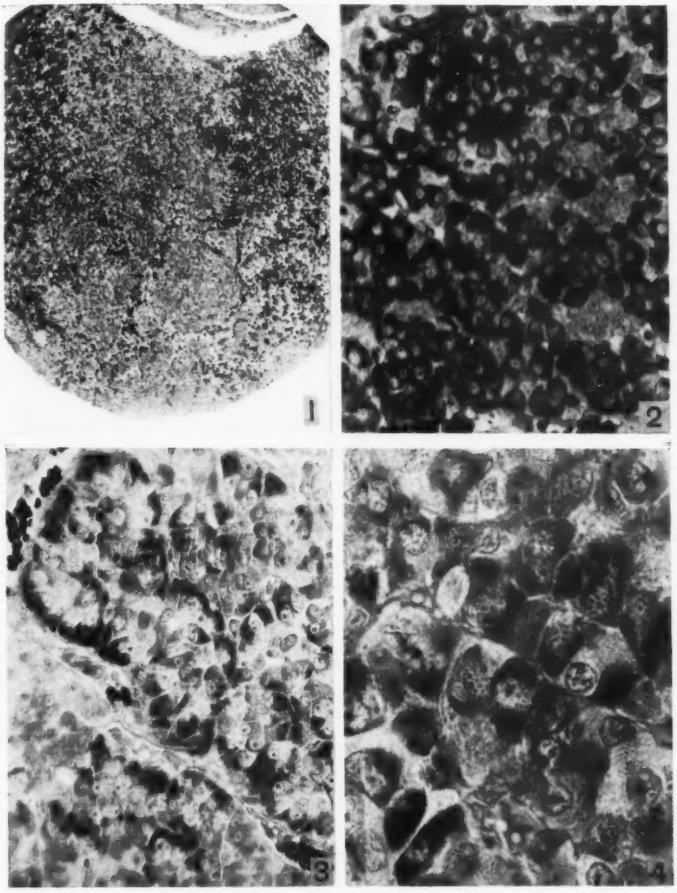


Fig. 1. Low power view of mid-portion of a frontal section of the anterior pituitary. The central area (gray) of basophils and chromophobes is the zone tuberalis which divides the pars anterior into right and left halves. The majority of the cells in the latter are ordinary acidophils (dark). Anestrous *cat 4A*, 10-25-37. Fig. 2. Medium power view (frontal section) of area from one wing of anterior lobe. Ordinary acidophils are dark and clusters of small chromophobes are gray. Anestrous *cat 8A*, 12-6-37. Fig. 3. Medium power view (frontal section) of a peripheral area adjacent to the zona tuberalis showing the characteristic alveolar pattern of the carmine cells (dark). Ordinary acidophils are also present (gray). *Cat. 1M*, 1 hr. 40 min. after mating, 6-1-37. Fig. 4. Oil immersion view of deeper lying area from the same pituitary as fig. 3 showing the distinctly granulated carmine cells (dark) scattered among the more homogenous, ordinary acidophils (gray). (Appropriate filters were used in each instance to achieve the desired contrast between the variously stained cells for purposes of photographic clarity.)

philic cells with relatively few chromophobes (fig. 1). Basophils were encountered only rarely in the lateral regions or wings (as seen in frontal sections) which were made up of ordinary acidophils with interspersed clusters of very small chromophobes (fig. 2).

Proestrous cats. Early proestrus can be recognized most accurately by a study of

serial sections of the ovaries. A cat is thought to be in the proestrous condition when the growing follicles reach a diameter of from 1 to 1.5 mm. (8). Toward the end of proestrus the ovaries cannot be distinguished from those of estrous animals, and estrus can be determined best by the physiological response of the animal. Accordingly,

TABLE 1. PITUITARIES OF ANESTROUS CATS

No.	Date of autopsy	Extent of carmine cell reaction	No.	Date of autopsy	Extent of cell reaction
1A	10/25/37	0	8A	12/ 6/37	0
2A	10/25/37	0	9A	12/ 9/37	0
3A	10/25/37	0	10A	12/ 9/37	0
4A	10/25/37	0	11A	1/ 4/38	0
5A	3/ 1/39	0	12A	2/10/38	0
6A	3/ 1/39	0	13A	2/10/38	0
7A	12/ 6/37	0	14A	4/22/38	0

there comes a period when it is impossible to distinguish histologically between a late proestrous and an estrous follicle, since significant changes in the cumulus, granulosa and germinal vesicle are initiated only several hours after mating or occasionally in atresia. With 1 exception (1P), the 7 cats which make up this group (table 2) were found to have ovarian follicles measuring 1 mm. or more in diameter.

TABLE 2. PITUITARIES OF PROESTROUS CATS

Number	Date of autopsy	Extent of carmine cell reaction	Sexual behavior
1P[1]	12/14/37	+	0
2P[2]	2/27/39	+	0
3P	3/ 2/39	+	0
4P	3/ 2/39	+	0
5P	3/27/39	+	0
6P	5/25/37	+	±[3]
7P[4]	6/13/38	+	0

[1] Old adult, not in heat. The pituitary picture was characteristic of proestrus, but the loss of the ovaries prevented verification of the level of follicular development.

[2] This young female had never been in heat. Ovaries showed many atretic follicles. Fragmentation of the ova had occurred in many of these follicles. The largest follicles (1 mm. in diameter) were in the first maturation division. No changes characteristic of the normal activation of ovarian follicles had occurred in the cumulus.

[3] Sexual behavior indicated doubtful estrus.

[4] Appeared in heat, but would not mate. Glass rod stimulation resulted in post-coital behavior. Ovarian follicles showed atretic activation; some were in proestrous state.

For extent of +carmine cell reaction, see fig. 6.

Carmine cells were present in small numbers in all 7 pituitaries. The appearance of carmine cells is therefore coincident with the attainment by the follicles of a size of 1 mm. or more in diameter.

The carmine cells appear first on the periphery of the anterior pituitary gland in those areas of chromophobic cells which lie lateral and immediately adjacent to the zona tuberalis (fig. 6). The areas occupied by the carmine cells thus constitute two poorly-defined superficial strips which converge along the anterior periphery of the gland and become less well-defined as they extend ventrally. At their extreme ventral extension the cells usually become more scattered and mingle irregularly with the basophils and chromophobes of the zona tuberalis. Some evidently arise by the granulation of small chromophobic cells; and in the early stages of granulation the specific

granules appear as a paranuclear crescentic mass. The carmine cells are seldom numerous at this stage and do not occur in the alveolar pattern and pyramidal form.

In proestrous cats there is marked degranulation of many basophils, and they become much smaller than the fully granulated cells. The zona tuberalis is contracted and not so distinctly set apart from the lateral acidophilic wings. This change in spatial pattern is conspicuous, and the apparent invasion by acidophilic cells seems to be due to changes in the volume of the zona tuberalis rather than to the transformation of the cells of this region into acidophils.

Estrous cats. The 6 animals included in this group (table 3) were isolated from the male and under continuous observation until they were autopsied at their first

TABLE 3. PITUITARIES OF ESTROUS CATS

Number	Date of autopsy	Extent of carmine cell reaction	Sexual behavior
1E	2/24/38	++	In 2nd day of heat. Good treading response when tapped at base of tail. R. ovary, 1 follicle; l. ovary, 3.
2E	3/14/38	++	In 2nd day of heat. Typical estrous behavior. R. ovary, 4 follicles; l. ovary, 1.
3E	3/15/38	+++	In 3rd day of heat. Good treading response; typical estrus. Rt. ovary, 4 large follicles; l. ovary, 1.
4E	3/30/38	+++	In 2nd day of heat. Good treading response; typical estrus. R. ovary, 1 follicle; l. ovary, 2.
5E	3/ 3/38	++	In heat. Treading response. Marked post-coital movements after stimulation of cervix uteri with glass rod. Autopsied 10 minutes after stimulation.
6E	4/ 1/38	see fig. 8	In 3rd day of heat. Maximum heat reaction. R. ovary, 4 follicles; l. 2.

For extent of ++ to +++ carmine reaction see fig. 7.

6E is an exception to the general rule and showed a much more extensive carmine-cell reaction than ordinary.

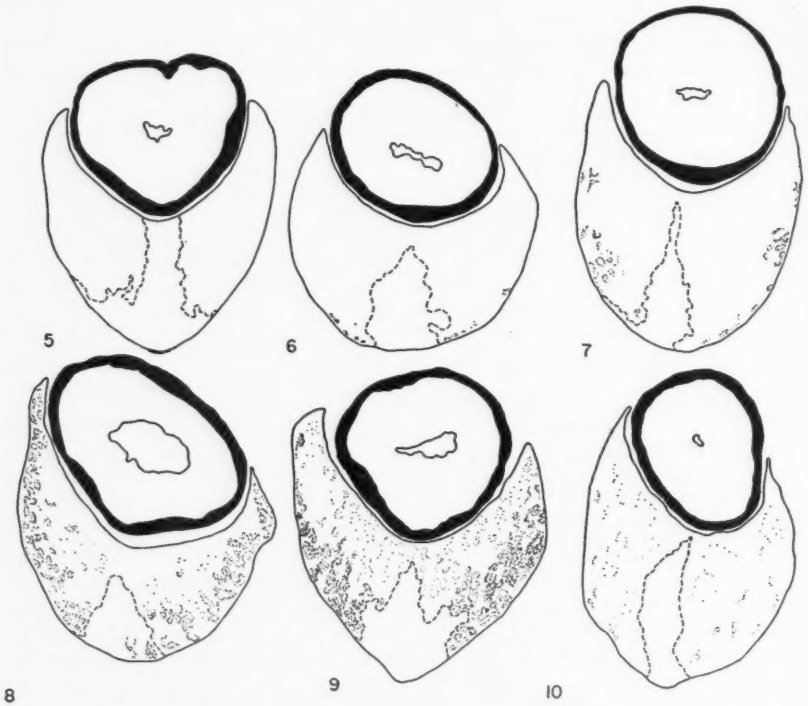
All cats were autopsied during the first estrus in which they were observed in 1938.

estrus. These experimental conditions obviated difficulties in the interpretation of the pituitary histology because of possible complicating factors such as a previous pseudopregnancy, pregnancy or recurrent estrus. The pituitaries of these animals, with the exception of *6E*, showed relatively small advances beyond the proestrous stage in the carmine-cell picture (fig. 7). The areas of carmine cells, which were somewhat more extensive than those seen in the proestrous animals, spread peripherally from the lateral margins of the zona tuberalis well into the wings of the anterior pituitary (as viewed in frontal section). Some carmine cells were partially granulated and others fully granulated, but only in rare instances were the alveolar patterns fully developed.

The carmine-cell reaction in *6E* was very marked, being comparable to the level reached 2 hours after mating. The carmine reaction, which was found completely around the external surface of the gland, also extended centrally for a considerable distance leaving an unmodified zone adjoining the pars intermedia. Typical alveoli were numerous, especially near the surface of the gland, but the cells were still scattered in the more central portions of the pituitary substance. The sexual behavior pattern of *cat 6E* was excessively libidinous. When tapped at the base of her tail she treaded vigorously and then rolled repeatedly even after such minimal stimulation. She did not utter the mating cry, but her behavior otherwise simulated closely the postcoital response. An examination of the ovarian follicles revealed no reaction in the cumulus beyond that obtained within 6 hours after mating or in other unmated estrous cats.

Mated cats. Twelve cats were autopsied at various intervals during the first 24

hours after mating and glass rod stimulation of the cervix uteri or after artificial stimulation alone. A detailed account of the matings and of the reactions which take place in the pituitaries and ovaries is given in table 4. A high correlation is seen between the extent and character of the carmine-cell reaction in the pituitary, the degree of activation of the ova and the sexual behavior pattern of the animal at and after coitus.



DIAGRAMMATIC OUTLINES OF FRONTAL SECTIONS OF THE PITUITARY illustrating the changes in number and distribution of the carmine cells (black dots). The central area, in the pars anterior (delineated by broken lines) is the zone in which basophils and chromophobes predominate. The pars intermedia, in solid black, invests the infundibular process.

Fig. 5. Anestrous: no carmine cells. Fig. 6. Proestrous: few peripheral carmine cells. Fig. 7. Estrous: increased number and distribution of carmine cells. Fig. 8. One hour and 40 minutes after mating: greatly increased distribution and number of carmine cells. Fig. 9. Five hours and 15 minutes after mating: regarded as maximum reaction. Fig. 10. Fourteen hours after mating: advanced degranulation of the carmine cells.

The carmine-cell reaction as early as 1 hour and 40 minutes after mating (*1M*) is very striking (fig. 8). The characteristic alveolar grouping of carmine cells (fig. 3), which is found about the entire periphery of the anterior pituitary, except in the region of the zona tuberalis, extends into the substance of the gland almost halfway toward the residual lumen. Beyond this zone of alveolar grouping the carmine cells are scattered and appear to be distributed more or less uniformly throughout the remainder of the gland (fig. 4). Many of the cells are fully granulated and others are in various intermediate stages of granulation.

About 4 hours after mating degranulation begins in the peripheral alveoli, a region in which granulated cells occur in estrous animals. Additional carmine cells develop deeper within the gland at this time. The maximum extent of the carmine reaction is observed about 5 hours after copulation (3*M*). Typical alveolar groups of carmine cells were found throughout the entire pars anterior of this gland (3*M*), while progressive degranulation was noted in the periphery of the pituitary (fig. 9).

TABLE 4. PITUITARIES OF CATS AUTOPSIED DURING THE FIRST 24 HOURS AFTER MATING AND/OR STIMULATION OF THE CERVIX UTERI WITH GLASS ROD

Cat number	Date of autopsy	Hours after mating and/or stimulation	Extent and distribution of carmine-cell reaction and degranulation[1]	Condition of ovarian follicles and ova	Sexual behavior before, during and after mating
1*M*	6/ 1/37	1¾	See fig. 8.	Estrous follicles.	Typical estrous behavior during mating. Post-coital response intense. Mated 4 times; glass rod 4 times within 20 minutes.
2*M*	7/11/38	4	See fig. 8. Initial degranulation.	Not examined.	Mated 3 times with typical estrous behavior and post-coital response.
3*M*	6/11/38	5¼	See fig. 9. Initial degranulation.	Estrous follicles.	Mated 2 times; glass rod 2 times within 20 minutes. Characteristic estrous behavior during and after mating.
4*M*	3/ 6/39	6⅝/12	Slightly more advanced than conditions in fig. 7.	Large cystic follicles in both ovaries.	Glass rod 4 times. Typical screaming after insertion of glass rod, but only abortive rolling after removal of rod. Behavior pattern not characteristic of a highly estrous animal.
5*M*	4/ 9/37	6¾	See fig. 9. Peripheral degranulation.	3 estrous follicles; early corona radiata.	Mated 2 times; glass rod 2 times within 15 minutes. Characteristic estrous behavior during and after mating.
6*M*	3/ 2/39	10	See fig. 9. Peripheral degranulation complete.	Cumulus reaction almost complete.	Glass rod 6 times in 30 minutes. Characteristic estrous behavior during and after rod.
7*M*	3/ 6/39	10	See fig. 9. Peripheral degranulation complete.	First maturation division.	Glass rod 4 times. Tossed spontaneously before stimulation. Typical post-coital movements after removal of rod.
8*M*	5/31/39	12½	See fig. 9. Patchy degranulation throughout gland.	Distinct tetrad formation.	Mated 5 times, screamed and treaded during mating; tossed and rolled characteristically post-coitum.
9*M*	6/14/39	14	See fig. 10. Progressively increasing degranulation beginning with 14 hours, and almost complete degranulation at 24 hours.	Tetrads compact.	Mated 7 times in 40 minutes. Characteristic coital and post-coital estrous behavior.
10*M*	1/25/39	18		First maturation spindle.	Mated once; refused second mating. Mild cry during coitus; not much treading; limited post-coital movements. Left with male 12 hours. Out of heat 24 hours after mating.
11*M*	3/ 9/39	22½		Second maturation spindle.	Arched back and treaded moderately in response to tapping at the base of the tail. Treaded and howled with insertion of rod but did not scream. Scratched vigorously behind left ear and rubbed against furniture but did not roll or toss about post-coitum.
12*M*	2/ 1/39	24		Second maturation spindle.	Mated 3 times. Treaded and cried moderately during mating. Post-coital movements typical of a good estrous state.

[1] Figures 8, 9 and 10 represent diagrammatically the extent and distribution of the carmine reactions in cats 1*M*, 3*M* and 9*M* respectively. In all other instances these figures are used to indicate the approximate level of the carmine reaction but the degree of degranulation varies from the figures as noted.

The carmine-cell reaction in *cat 4M* was not as extensive as that observed in other cats before and after this time. Initial activation of the pituitary had apparently occurred, but it was not sufficient to induce even the early changes in the cumulus observed by this time after mating. As noted in table 4 the post-coital movements of this animal were not typical.

Mating stimulated the pituitaries in the remaining animals of this group success-

fully, and a definite, chronological sequence of changes was observed in the glands as well as in the follicular cells and ova. As noted above, there was a significant correlation between the progress of events in these two glands.

It is difficult to estimate the total extent of the carmine-cell reaction after the 6th hour, since the degranulation of the peripherally distributed cells is marked and many of the carmine cells are completely degranulated. In some instances the cells are reduced in size to that of the chromophobes. Although the alveolar groupings may still be recognized here and there, they disappear for the most part so that the displaced standard acidophils resume their irregular pattern of distribution. In some of the carmine cells the vestiges of the carmine reaction persist longer than in others. The reaction may be regarded as virtually over by 14 hours (fig. 10), although evidences of it are not entirely lacking as late as 24 hours. Tetrads are distinctly seen in the ovum at the 12th hour, and by the 20th hour the preovulatory changes in the follicles are complete (first polar body and second maturation spindle). The period of extensive degranulation in the carmine cells of the pituitary is coincidental with these preovulatory phenomena of maturation.

DISCUSSION

The carmine cells appear in the pituitary coincidentally with the proestrous growth of ovarian follicles. These cells increase in number with the appearance of estrous behavior and reach their full development cytologically during the first few hours after mating. The early hours of the post-coital period are also marked by a significant change in their pattern of distribution. These observations are in accord with earlier ones made on the rabbit (1, 2).

The cytological significance of the carmine cells is not clear. It is obvious that they arise, at least in part, from small non-granular cells and after degranulation resume the same appearance. The possibility of the transformation of acidophils staining with orange G into those staining with azo-carmine is not entirely excluded. However, transitional stages between the two tinctorial types of acidophils have not been recognized. This may be due to the limitations of the technical methods employed, although comparable differential staining may be obtained by fixing and staining methods other than those followed in this study. A detailed analysis of the reactions of the two types of acidophils with a different fixation and a variety of selective staining methods has been published (19) and confirms the view that the differential staining obtained is not a vagary of the method, but represents some physicochemical difference between the two types of acidophils.

The precise physiological significance of the carmine reaction is still sub judice. Its temporal relation to known physiological changes in the female reproductive system strongly suggests that it is concerned with the production and release of a specific gonadotropic hormone. Since ovulation is not induced by FSH alone, but LH is required in addition to elicit this response in the rabbit and cat, it would be natural to associate the carmine reaction with the secretion of LH. Cumulative evidence indicates that the basophils are concerned with the secretion of FSH (20).

The behavior of the carmine cell has not yet been determined for the entire period of gestation. Our present incomplete data on the first 3 weeks suggest that after ovulation the cell practically disappears for a brief period, reappears while the ovum is traversing the oviduct, and disappears again when the ovum is undergoing implantation in the uterus. More positive statements can be made regarding the situation from the 4th week until the termination of pregnancy. During this period there is a progressive and striking increase in the number of carmine cells until they dominate the cytological picture at term. Our scattered observations on the postpartum

period, with and without lactation, are too few to warrant any conclusions concerning the changes in the number of carmine cells associated with these important physiological states. However, the presence of the carmine cell in great numbers at the end of gestation and the beginning of lactation can hardly be without significance.

SUMMARY

After fixation in formol-sublimate and staining with Heidenhain's azan, carmine-staining acidophils can be demonstrated in the pituitary gland of the female cat during certain phases of the reproductive cycle. This technic also differentiates the usual acidophils, which stain with orange G, and the basophils. The carmine cells are absent in anestrus, sparsely present in proestrus and increased in number during estrus. Their pattern of distribution in the pituitary changes characteristically after mating and their greatly increased numbers reach a maximum within 5 to 6 hours. Some degranulation occurs as early as 4 hours post-coitum and this becomes more pronounced after 6 hours. The carmine reaction is virtually over at 14 hours.

This temporal sequence of events in the pituitary gland parallels closely the cytological changes which occur in the mature ovarian follicle. At 6 hours post-coitum one can distinguish the initial changes in the cumulus and mural granulosa which are indicative of follicular activation. The changes in the follicular epithelium become progressively more pronounced thereafter, while the first evidences of the maturation of the ovum are observed at 10 hours. Tetrads are free in the cytoplasm at 12 to 14 hours. The first maturation spindle is present at 18 hours; and the first polar body and second maturation spindle are found at 22 to 24 hours.

It is concluded that the function of the carmine cell is directly concerned with the gonadotropic activity which is rapidly initiated in the pituitary gland by the act of mating. Certain theoretical considerations suggest that it is most closely concerned with the secretion of LH. It is also probable that the carmine cell is intimately associated with certain other reproductive functions of the cat which occur in pregnancy and lactation.

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THE RELATIONSHIP BETWEEN THE SECRETORY CELLS OF THE PARS NERVOSA OF THE HYPOPHYSIS AND CLASSICAL NEUROGLIA

MERVYN GRIFFITHS[1]

By the use of methods which stain oligodendroglia and microglia, Bucy (1, 2) demonstrated the presence of characteristic neuroglial cells in the infundibular stalk and process of the bovine pituitary. These cells he termed pituicytes. The pituicytes vary greatly in shape and size but have two characteristics in common: firstly, the cell body is drawn out into a variable number of cytoplasmic processes, one or more of which are attached by foot plates to connective tissue (either in the form of pia, septa, or that around blood vessels), and secondly, the cells are all well impregnated by Penfield's (3) second modification of the del Rio-Hortega (4) silver carbonate technic for oligodendroglia. This modification stains both oligodendrocytes and microglia.

The pituicytes are distributed diffusely throughout a rich plexus of unmyelinated nerve fibers which make up the main mass of the gland. The supraoptic and to some extent the paraventricular nuclei are the principal sources of the fibers of the tractus hypothalamico-hypophyseus. Recently, Rasmussen (5) has studied the nerve supply of the human pars nervosa and gives a very full bibliography pertinent to the innervation of the hypophysis in general.

It has been stated by a few authors (6, 7, 8) that neurocytes occur in the pars nervosa while many other authors have denied their occurrence. Ibáñez (9) reinvestigated this question and showed that neurocytes do not occur and that the cells are glial. Ibáñez discusses the relevant literature and the concensus is that neurocytes are absent.

Griffiths (10, 11) has shown that pituicytes occur in the partes nervosae of all the classes of vertebrates and that they are fairly similar in morphology throughout the series except in Elasmobranchs where they have very few short cytoplasmic processes or none.

De Lawder Tarr and Geiling (12) Geiling (13, 14), Geiling and Oldham (15) and Fisher (16) have very thoroughly discussed the question of the origin of the posterior lobe hormones and the data gained from their experiments prove that the pars nervosa itself is responsible for the elaboration of the pressor-antidiuretic and oxytocic principles, and not the pars intermedia as has hitherto been widely held. The more recent work of Oldham, McCleery and Geiling (17) and Oldham (18) on the anatomy and pharmacology of the hypophyses of the manatee and armadillo respectively, supports this concept.

The work, recently reviewed (19), of Fisher, Ingram and Ranson, and that of Gilman and Goodman (20), proves that the pars nervosa is a true endocrine gland in respect to water metabolism; the antidiuretic principle preventing undue water excretion. Gilman and Goodman have shown that there is an increase in secretion of antidiuretic hormone by the rat pars nervosa when the animals are dehydrated. Gersh (21) has shown that the nervosa glia is responsible for this secretion. By impregnation with osmium he demonstrated lipoid granules or droplets in the bodies and processes of the cells. These, so revealed, were found to be markedly hypertrophied, and their numbers increased when the animals were dehydrated. On hydration the number and size of the cells were found to have returned to within the limits of variation exhibited by hydrated controls.

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[1] 1851 Exhibition Science Research Scholar. Permanent address: Department of Zoology University of Sydney, Australia.

Additional evidence presented by Griffiths (10, 11) shows that the nervosa glia is responsible for the elaboration of the posterior lobe hormones.[2] However, Rasmussen (5), impressed by the extraordinary richness of the nerve supply to the pars nervosa, which to him seemed out of all proportion to the cellular content, is inclined to think that the hormones produced by the posterior lobe are liberated without the intervention of secretory cells in the usual sense, as in the humoral mechanism associated with the sympathetic nervous system. Griffiths (10, 11) has presented evidence elsewhere, which disproves this view.

In his original description of pituicytes, Bucy raised the question of the relationship between these and the neuroglial cells of the nervous system. The pituicytes are well impregnated by methods for microglia and oligodendroglia but are quite unlike these morphologically. Bucy remarks, however, that a few with single vascular process and foot plate resemble astroblasts. Geiling and Robbins (22) state that in the white whale, *Delphinapterus leucas*, the pituicytes are like the ependymal spongioblasts of the embryonic nervous system or the cells of ependymal tumours.

In view of the confusion of opinion in the literature, this study[3] was undertaken to establish a clearer concept of the relationship between pars nervosa glia and that of the central nervous system, by a comparison of their morphologies, geneses and cytoplasmic inclusions.

METHODS

Genesis of pituicytes was studied in 1-day-old to 25-day-old chickens, and morphology in adult fowls, dogs and rats. The cells were impregnated with del Rio-Hortega's (4) silver carbonate method for oligodendroglia (S.C.O.) and with Penfield's (3) modification of this technic (S.C.O.M.)

The use of Spanish silver methods generally demands that sections be cut by the freezing method, but the writer finds that good silver impregnations can be made with paraffin sections, which is advantageous when using pieces of tissue as small as the partes nervosae of day-old chickens. The glands were fixed in Cajal's formalin-ammonium bromide, dehydrated, de-alcoholized in xylol, embedded in paraffin, sectioned serially, and passed into xylol. The now separate sections were alcoholized, gradually hydrated, washed in several changes of distilled water, and then treated as sections obtained by the freezing method.

Cytoplasmic inclusions were studied in cats, dogs and fowls by means of del Rio-Hortega's (23) silver carbonate methods for gliosomes (S.C.G.) and by means of the usual S.C.O. and S.C.O.M. technics. For the S.C.G. method, frozen sections were imperative. Lipoid inclusions were also studied in rats and fowls by Gersh's osmic acid method.

Nuclear structure was studied in material fixed in Schaudinn's fluid and stained with Heidenhain's iron hematoxylin.

Morphology

The pars nervosa of the chicken develops as a few irregular outgrowths of the infundibulum, each containing an extension of the infundibular cavity. According to de Beer (24) and Atwell (25) these evaginations of the infundibular cavity are

[2] Since submitting the manuscript for publication, the writer noticed that Wang (40) recently published evidence supporting the concept of the secretory nature of pituicytes.

[3] The work was carried out in the Laboratory of Neuropathology of the Montreal Neurological Institute, and the writer takes this opportunity to express his gratitude to the Director of the Institute, Dr. Wilder Penfield, for his stimulating advice and criticism, and for extension of laboratory facilities. The writer also wishes to acknowledge his indebtedness to Dr. I. Gersh of the Department of Anatomy, The Johns Hopkins University, for information concerning the details of his osmic acid method for pituitary glia.

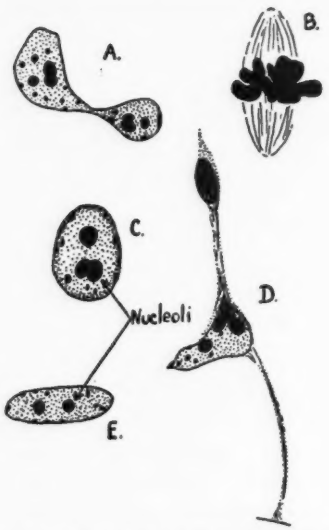

Fig. 1. Nuclei of pituicytes and spongioblasts in adult fowls and chickens. A, nucleus of a spongioblast in an adult gland which appears to be dividing amitotically. B, mitotic figure in spongioblast of 25-day-old chicken. C, and E, oval and elongated types of pituicyte nuclei. D, apparent separation of a nuclear bud from its parent nucleus in pituicyte. (Not drawn to scale.)

present as early as the seventh or eighth days of incubation. The writer's observations are in agreement with this. From the primary outgrowths, numerous secondary outgrowths develop, some of which possess a derivative of the infundibular cavity, while others are solid. Connective tissue sheaths surround and invade the outgrowths and impart the lobulated appearance characteristic of the sectioned adult gland. The blood vessels occur as splits between the laminae of connective tissue.

In S.C.O. and S.C.O.M. preparations of the adult gland, the infundibular cavity and its derivatives are seen to be lined by an epithelium formed of supportive spongioblasts, the cell bodies, proximally, bordering on the lumina and the distal processes ending in foot plates on the connective tissue sheaths and septa (fig. 3) just as do supportive spongioblasts in the embryonic central nervous system. For comprehensive accounts of the morphology and genesis of the central nervous system glia, see Penfield (26), Jones (27), Kershman (28). The pituicytes are distributed indiscriminately throughout the outgrowths. Their cytoplasm is finely granular and without fibers; practically all of them possess one or more vascular processes ending in foot plates on connective tissue. These foot plates, as can be seen in figures 4 and 13D are funnel-shaped and morphologically indistinguishable from the pial and vascular attachments of astrocytes, but beyond this point the resemblances to astrocytes are not marked. Pituicytes may have long, slender vascular processes or short, thick ones; the cell bodies may have little cytoplasm or may have a large area surrounding the nucleus; the number of cytoplasmic processes varies from very few to many. Fibrous and protoplasmic astrocytes from a given region of the brain, on the other hand, have a comparatively constant morphological configuration and are not readily impregnated by the silver carbonate methods that stain pituicytes well. Many of the pituicytes, however, with one or two long, slender processes, bear a remarkable resemblance to the subpial astroblasts depicted by Kershman (28) in the spinal cords

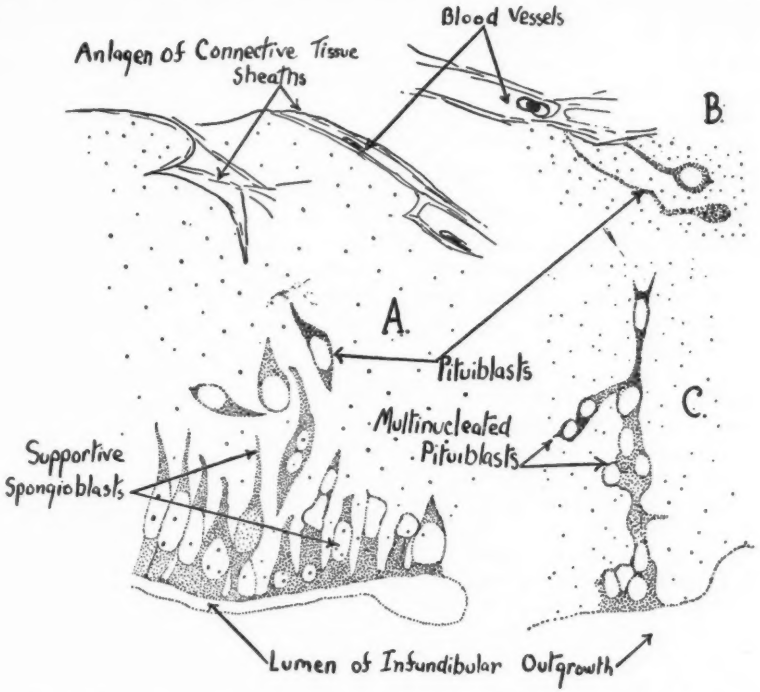

Fig. 2A. PITUIBLASTS OF DAY-OLD CHICKEN, QUITTING SPONGIOBLAST EPITHELIUM. B, pituiblasts with vascular attachments. C, cytoplasm of multinucleated pituiblast dividing to form individual pituicytes. (Not drawn to scale.)

of 8 and 16-week human embryos. These astroblasts, moreover, are readily impregnated by the S.C.O.M. technic.

Morphologically, then, pituicytes are related to astrocytes, and inasmuch as they do not possess fibres in their cytoplasm, to protoplasmic astrocytes and not to oligodendrocytes or microglia as these latter have no vascular processes and foot plates.

The nuclei of avian pituicytes and supportive spongioblasts vary in shape from a rounded oval type (fig. 1C) to an elongated form (fig. 1E), the chromatin occurring as small, irregularly distributed granules and as larger nucleoli. The number of nucleoli varies from one to as many as four. Frequently the nuclei assume shapes and configurations which, in the writer's opinion, indicate the occurrence of amitotic division; some nuclei develop small buds (fig. 5) which in many cases may be seen to be almost disconnected from the parent nucleus; others may be seen dividing into two nuclei of equal size (fig. 1A). In figure 1D is depicted a nuclear configuration which the writer interprets as showing a nuclear bud and parent nucleus after separation. The pyknosis of the smaller nucleus and the accumulation of chromatin in the parent nucleus on the side nearest the bud supports this view.

Genesis

In sections of the partes nervosae of young chickens, stained by the S.C.O. method, the lumina of the infundibular stalk and outgrowths are seen to be lined by

an epithelium of supportive spongioblasts (fig. 6 and 2A). From this epithelium elongated, bipolar spongioblasts may be seen migrating out towards, and implanting foot plates upon, the peripheral connective tissue (fig. 2B) or that invading the outgrowths. These cells which have quitted the ependymal epithelium, the writer proposes to call pituiblasts. The pituiblasts for the most part are bi- and monopolar elements. Having arrived at their definitive positions, they become pituicytes by further development of cytoplasmic processes and vascular feet. Similarly, astroblasts are derived from supportive spongioblasts, the foot plates of which end on the pial connective tissue or on the blood vessels invading the nervous tissue. Oligodendrocytes, however, are derived from those apolar and migratory spongioblasts that never develop vascular attachments, while microglia, the remaining argentophil element of the interstitial tissue of the brain, is mesodermal in origin (29, 30). Thus on the evidence of development, pituicytes are closely related to astrocytes and to a certain degree are allied to oligodendrocytes. They bear no relationship to microglia.

The process of migration of spongioblasts from the pars nervosa ependyma continues even in the adult bird. In addition to displacement of single spongioblasts, multinucleated masses of argentophil cytoplasm quit the epithelium and migrate into the gland. The masses possess cytoplasmic processes, the distal members of which end in foot plates on the connective tissue sheaths. The writer proposes the name of multinucleated pituiblasts for these masses. Their mode of migration and morphology are illustrated in figures 2C, 7 and 13F. The multinucleated pituiblasts may persist in their definitive positions as such, or the cytoplasm may finally divide, resulting in the formation of individual autonomous pituicytes. This process is illustrated in figure 2C.

In the armadillo pars nervosa, Oldham (18) reports the presence of groups of cells or multinucleated masses of cytoplasm in which cell boundaries cannot be distinguished, the nuclei of which are far larger than those of the typical pituicytes of this species. Numerous intermediate types occur between these and the groups with large nuclei. The writer thinks it possible that these groups correspond to the multinucleated pituiblasts of the fowl. Ibáñez (9), using the Golgi technic, also reports the presence of apparent groups of pars nervosa glia in young dogs and cats, but attributes the syncytial appearance to deposition of silver between closely arranged individual cells.

In the gland of the young chicken, the spongioblasts and pituiblasts multiply by mitotic division (fig. 1B, 8 and 9). This is interesting in view of the fact that in the developing central nervous system, Kershman (28), by the use of the S.C.O.M. technic has been able to demonstrate mitosis in cells identifiable as migrating spongioblasts. No evidence of the amitotic division of pituiblasts, noticed in the adult, was found in the young chicken.

Cytoplasmic Inclusions

The work of Nageotte (31), Mawas (32), Fieandt (33), Achúcarro (34), Cajal (35), Penfield (36) and del Rio-Hortega (23) has amply proven the existence of characteristic cytoplasmic inclusions in astroblasts, astrocytes, oligodendroblasts and oligodendrocytes. These inclusions are known as gliosomes and are markedly distinct, ovoid or spherical bodies of variable size which occur in the cell bodies, cytoplasmic processes and vascular feet. The morphological relations of gliosomes in astrocyte and oligodendrocyte are shown in figures 13A, B and C. Gliosomes are not found in microglia. Del Rio-Hortega (23) has devised a specific technic for these bodies whereby they are energetically impregnated whilst the cell bodies and expansions are faintly outlined. Too heavy impregnation of the cells results in the

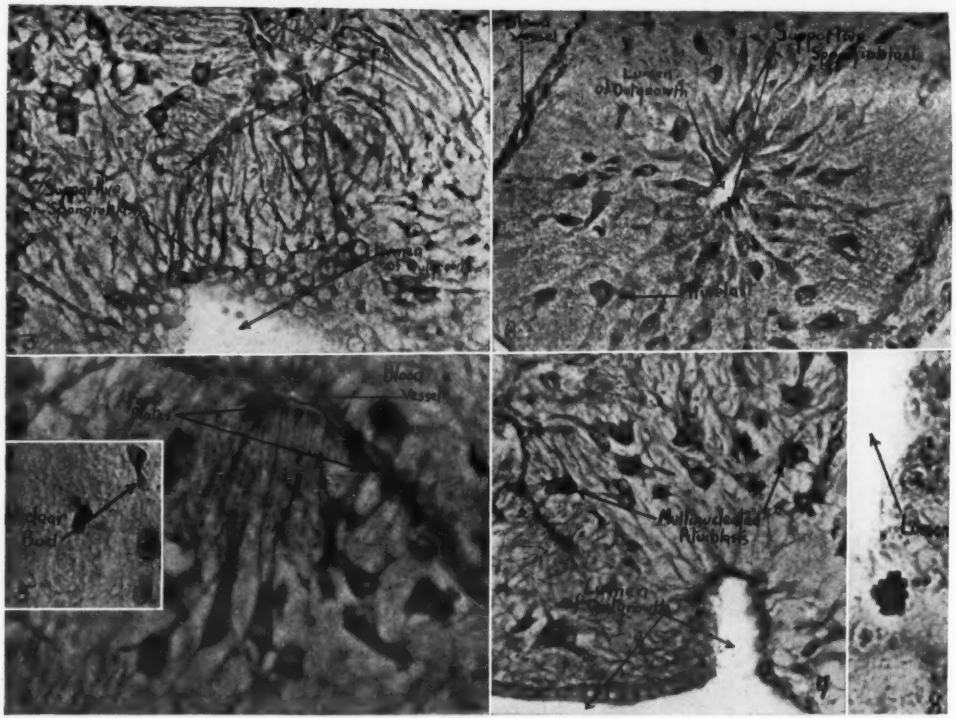

Fig. 3. ADULT FOWL PARS NERVOSA. Portion of an infundibular outgrowth showing epithelium of supportive spongioblasts about the lumen, and attachments of the distal processes of the spongioblasts to a connective tissue sheath (C.T.S.). (S.C.O. ×485.) Fig. 4. PITUICYTES, SHOWING THEIR VASCULAR PROCESSES AND ATTACHMENTS (FOOT PLATES) TO A BLOOD VESSEL IN A CONNECTIVE TISSUE SHEATH. (S.C.O. ×875.) Fig. 5. ADULT FOWL. Pituicyte nucleus dividing amitotically by formation of a nuclear bud. (Heidenhain's iron hematoxylin ×835), Fig. 6. DAY-OLD CHICKEN. Section of an infundibular outgrowth. The pituiblasts can be seen migrating away from the spongioblast epithelium towards the peripheral blood vessels and connective tissue. (S.C.O. ×490.) Fig. 7. ADULT FOWL. Multinucleated pituiblasts migrating away from the ependymal epithelium lining the lumen of an infundibular extension. (S.C.O. ×470.) Fig. 8. FIFTEEN-DAY-OLD CHICKEN. Mitotic figure in a supportive spongioblast. (Heidenhain's iron hematoxylin ×1235.)

gliosomes being obscured or obliterated. Since the presence of gliosomes is characteristic of neuroglia it is obviously necessary in a comparison of pituitary glia with that of the central nervous system, to ascertain whether or not, pituicytes possess such inclusions. The application of S.C.G., S.C.O. and S.C.O.M. methods demon-

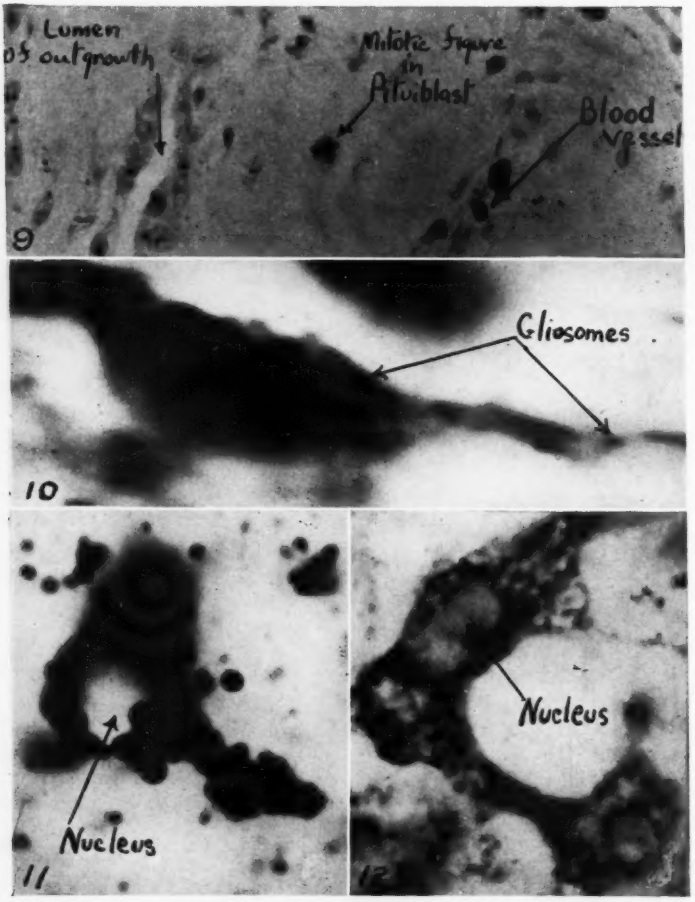

Fig. 9. TWENTY-FIVE-DAY-OLD CHICKEN. Pituiblast which has just migrated away from the ependymal epithelium, dividing by mitotis. (Heidenhain's iron hematoxylin ×760.) Fig. 10. DOG PITUICYTE SHOWING ROUNDED GLIOSOMES. (S.C.O.M. ×4400) Fig. 11. LIPOID DROPLETS IN A RAT PITUICYTE. (Gersh's osmic acid method ×2000.) Fig. 12. RAT PITUICYTE. (S.C.O.M. method ×2,200.)

strated in mammalian and avian pituicytes, clear-cut, ovoid granules of varying sizes in the cell bodies and processes (fig. 13D, G, H and fig. 10). The granules have the same staining properties as those of brain neuroglia and because of the similarity of morphology and relations within the cell, the writer considers them to be completely homologous with neuroglial gliosomes, and will refer to them as such. Gliosomes also occur in pituiblasts (fig. 10E, F) as they do in neuroglia precursors.

A comparison of chicken and rat pituicytes stained by silver methods and by Gersh's osmic acid method showed that probably the osmophilic or lipoid inclusions are not gliosomes, the former being much larger than the latter. The lipoid droplets extend out into the cell processes and the outlines and relations of the cells thus re-

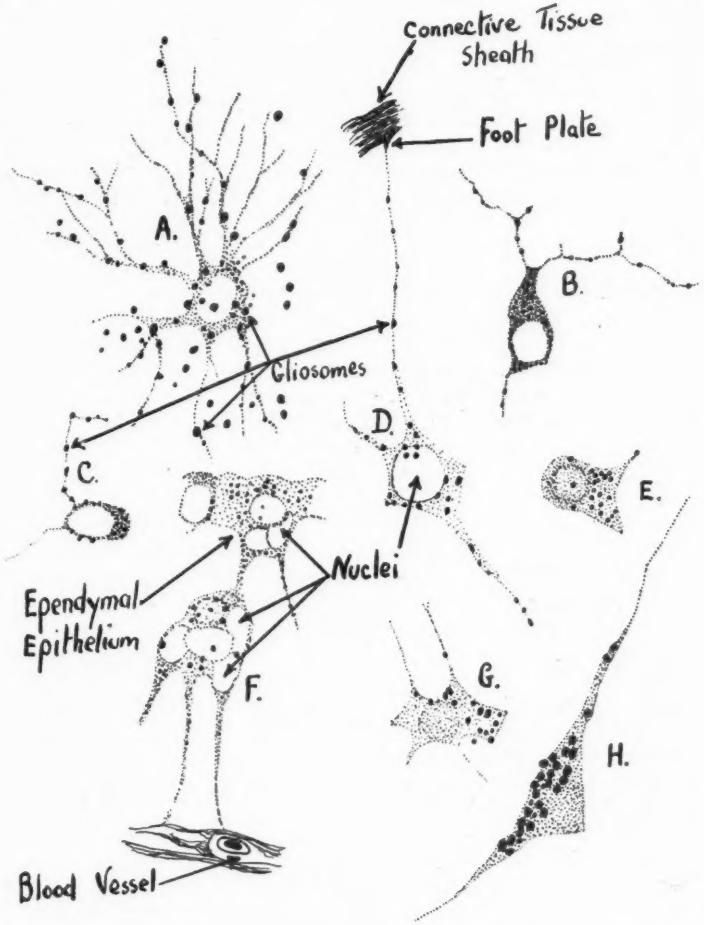

Fig. 13. Showing gliosomes in A, protoplasmic astrocyte (S.C.G.), cat. B, oligodendrocyte (S.C.O.), pig. C. oligodendrocyte (S.C.O.), fowl. D, pituicyte (S.C.O.), fowl. E, pituiblast (S.C.O.), day-old chicken. F, multinucleated pituiblast (S.C.O.), fowl. G, pituicyte (S.C.G.), cat. H, pituicyte (S.C.O.M.), dog. (Not drawn to scale.)

vealed, agree very well with those shown by silver (fig. 11, 12). Previous to Gersh, Parhon and Caraman (37) stained these lipoid droplets in the rat with sudan and nile blue. The same picture is obtained with these stains as with osmium, the cells presenting the same variations in shape and size, and possessing processes filled with lipoid droplets. These authors state that the cells can be so numerous that they appear

as syncytial groups. The writer has observed this in osmium preparations of the partes nervosae of rats dehydrated for 24 hours. Gersh mentions that not all the glial cells contain lipoid granules; many are relatively undifferentiated but rapidly acquire lipoid inclusions consequent upon the receipt of a dehydration stimulus. It is interesting to note in this connection that Lewis and Miller (38), after fixing in a modified Lane's solution and staining with Mallory's connective tissue stain, can also separate the secretory cells of the pars nervosa into two types. The use of silver impregnation, however, has not permitted the writer to make this distinction, the cells presenting the same staining reaction throughout the gland.

Further evidence in support of the nonidentity of gliosomes and the osmophilic inclusions is found in the fact that Gersh failed to find lipoid droplets in the central nervous system neuroglia. However, the question of the identity of gliosomes, lipoid droplets, and yolk-like granules observed by Lewis and Miller merits further investigation.

DISCUSSION

The fact that pituicytes possess gliosomes, and that they are demonstrated by the S.C.O. technic, relate them to oligodendrocytes as much as to astrocytes, but, as already indicated on morphological and developmental grounds, the pituicytes are closer to protoplasmic astrocytes. It appears, then, that the three groups constitute a closely interrelated morphological, but not functional, unit.

Nageotte (31) and Mawas (32) have attributed a glandular significance to astrocytes (at this time oligodendroglia had not been demonstrated). Achúcarro(34) has accepted and extended the hypothesis, considering it plausible that the interstitial cells constitute a gland of internal secretion. Cajal (35) and Hortega (23) have been far more reserved in their support of Nageotte's concept. The evidence for the claim is not conclusive being based mainly on the fact that the cells possess granules (Fieandt's gliosomes) which Nageotte considered to be similar to mitochondria and to resemble in their development, the secretion granules of gland cells.

Later studies (36, 39) have indicated that probably oligodendrocytes are actively associated with myelin secretion; the cells, laden with gliosomes, appearing in the brain and spinal cord at the time of myelination and not before. In addition, the gliosomes at this time are larger and more numerous than at any other stage of development. This type of secretory activity is hardly that envisaged by Achúcarro; however, the fact that the pituicyte group of the glial unit has endocrine functions, at the least places the concept of an endocrine function of astrocytes well within the realm of possibility.

SUMMARY

Pituicytes, like protoplasmic astrocytes and oligodendrocytes have a finely granular, nonfibrous cytoplasm, cytoplasmic processes, and typical ovoid gliosomes, but unlike those of oligodendroglia, many of the cytoplasmic processes form vascular and pial attachments, indistinguishable morphologically, from those of astrocytes.

The geneses of astrocyte and pituicyte are similar, both being derived from supportive spongioblasts. The term pituiblast is proposed for the displaced spongioblasts. In chickens both the spongioblasts and pituiblasts multiply by mitotis, but in the adult bird in which the migration of pituiblasts, away from the ependymal epithelium, continues, the pituiblasts and pituicytes appear to divide amitotically. In the adult, in addition to the usual migration of single spongioblasts, masses of nucleated cytoplasm quit the ependymal epithelium. These masses possess cytoplasmic processes and vascular attachments and have been termed multinucleated pitui-

blasts. They may persist as such in their definitive positions, or their cytoplasm may divide, and typical, independent pituicytes result.

Thus on grounds of morphology and development, pituicytes are closely related to protoplasmic astrocytes, and are related in less degree to oligodendrocytes. The three groups appear to form an interrelated glial unit.

Pituicytes possess rounded osmophilic lipoid inclusions which probably bear no relationship to gliosomes.

Silver impregnation does not admit of the separation of pituicytes into two groups as do osmic acid impregnation and staining by Mallory's connective tissue stain.

The fact that the pituicyte group of the glial unit has endocrine functions contributes to the plausibility of Achúcarro's hypothesis of the endocrine function of astrocytes.

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THE EFFECT OF CERTAIN EXPERIMENTAL CONDITIONS UPON THE THYROTROPIC HORMONE CONTENT OF THE ALBINO RAT[1]

C. W. TURNER AND P. T. CUPPS[2]

From the Department of Dairy Husbandry, University of Missouri
COLUMBIA, MISSOURI

IN A PREVIOUS PAPER (1) the thyrotropic hormone concentration in the AP of the male rat during growth and of the female during growth, pregnancy and lactation was reported. A higher concentration of hormone in the male was observed during all stages of growth, with the maximum in both sexes being reached during the period of rapid growth. In the estrous female the hormone concentration was low and continued so during the first half of pregnancy. There was evidence of an increase during the latter part of pregnancy and a further 40% increase during lactation.

At the present time relatively little is known concerning the factors playing a rôle in the regulation of the rate of secretion of the thyrotropic hormone aside from temperature (2, 3) and thyroidectomy (review, 4). It, therefore, was of interest to determine the relation of the gonadal and thyroid hormones to the thyrotropic content of the AP of both male and female albino rats.

EXPERIMENTAL

Procedure. Rats of both sexes weighing between 150 and 200 gm. were grouped and treated as follows: (*a*) castrated 20 days; (*b*) castrated 66 days; (*c*) castrated and injected daily with 40 R.U. of progynon-B[3] (estradiol benzoate) for 15 and 20 days; (*d*) castrated and injected with 200γ of oreton[3] (testosterone propionate) for 20 days; (*e*) castrated and injected with 0.03 mg. of thyroxin daily for 20 days and (*f*) thyroidectomized for 40 days and for 6 months. The body weights recorded in table 1 were obtained just prior to the killing of the animals.

The pituitaries of each group of rats were removed, weighed and frozen until assayed with day-old White Leghorn chicks according to the Bergman-Turner method (5) and the chick units computed from the equations presented. In each assay, chicks from 3 or 4 lots were used and the thyroids from a number of untreated chicks from each lot served as additional controls. In groups of chicks obtained weekly from October 1938 to August 1939, 3 lots of uninjected chicks (2 in April and 1 in August) appeared to have thyroids somewhat heavier than the normal controls. In table 1, the difference between the thyroid weight of the lot controls and the injected chicks of the same lots is presented.

OBSERVATIONS

To compare with the experimental data on the thyrotropic hormone content of

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the rat pituitary, data on normal male and female rats are presented (table 1). Weight groups in the range considered, presented in the previous report, are again included as well as certain additional weight classes. In the normal male rat, it will be noted that there is a rapid decline in chick units per gm. of pituitary tissue with increasing body weight due chiefly to an increase in weight of the pituitary. Per pituitary the thyrotropin content remains about the same.

The normal female rat pituitary shows a less definite trend in thyrotropic content with increasing body weight in the weight range considered. Possibly the average of the 4 groups represents a fair evaluation, 137 chick units (ch.u.) per gram and 1.369 ch.u. per pituitary.

Effect of gonadectomy. The removal of the gonads from both male and female rats appears to cause a significant reduction in the thyrotropic hormone content of the AP. In a group of males weighing an average of 152 gm., and with pituitaries weighing 6.11 mg. the thyrotropic hormone content was 0.995 ch.u. per pituitary and 163 ch.u. per gm. of pituitary following a castration period of 20 days. For a comparable weight group of normal rats the hormone content was 1.888 ch.u. per pituitary and 346 ch.u. per gm. This would indicate a reduction of about 47% from the normal. In a second group of males castrated 66 days with an average body weight of 187 gm. and a pituitary weight of 10.33 mg. there was a further marked decline in thyrotropin to 0.310 ch.u. per pituitary or 30 ch.u. per gm. of pituitary.

Two groups of female rats weighing 181 gm. were ovariectomized for 20 days. One group of pituitaries was injected into chicks at the rate of 3 mg., while the other lot was injected at the rate of 6 mg. per chick. The chick units per pituitary varied from 1.0888 to 1.159 with an average of 1.125 ch.u. and between 101 and 130 ch.u. per gm. of pituitary or an average of 116 ch.u. In comparison with the average normal females (1.369 ch.u. per pituitary) this represents a reduction of about 18%. The pituitaries from a group of females, castrated for 66 days, when injected at the 3 mg. level showed no appreciable amount of thyrotropin. From these data it would appear that there is some reduction in the thyrotropic content of both male and female rat pituitaries after 20 days of gonadectomy but the reduction becomes very marked after 66 days of castration.

Effect of estrogen. A group of male rats weighing 171 gm. was castrated and immediately injected with 40 r.u. of estradiol benzoate daily for 20 days. The pituitaries averaged 9.11 mg. being considerably larger than those of normal controls of comparable body weight. On the basis of ch.u. per pituitary there was a reduction from 1.888 to 1.039 or about 45% in the thyrotropin content. In the females castrated and given 40 r.u. of the same estrogen for 15 and 20 days, a marked increase (approximately 100%) in the pituitary weight was observed. When the pituitaries were injected at the 3 mg. level it was calculated that they contained an average of 1.079 ch.u. in comparison with an average normal of 1.369 or a reduction of about 21%. In a second group of female castrates injected for 15 days with the same dosage of estrogen, the average pituitary contained 1.762 ch.u. which is somewhat above the average normal but no higher than the 207 gm. weight group of normal females. When female castrate pituitaries were injected at a 12 mg. level they indicated a low thyrotropic content which was not in harmony with the other observations.

These observations appear to indicate that the injection of estrogen into male rats was unable to prevent the reduction of thyrotropin which occurs following castration. The data on the gonadectomized females, however, appears to indicate that estrogen was able to maintain approximately the level of thyrotropin present in the normal animal. Unfortunately, in the assays of these groups of pituitaries

TABLE 1. THE ALTERATION OF THE THYROTROPIC HORMONE CONTENT OF THE RAT PITUITARY BY CERTAIN EXPERIMENTAL PROCEDURE

No. and sex of rats in group	Rat body wt. ave.	Rat pituitary wt. ave.	Wt. pituitary injected per chick	Wt. of chicks, ave.	No. and sex of chicks in assay group	Chick[1] thyroid wt., ave.	ch.u.[2] in amount injected	ch.u. per gm. pituitary	ch.u. per pituitary	ch.u. per 100 gm. body wt. rat	Thyroid wt., difference, exp.-control	Treatment
	gm.	mg.	mg.	gm.		mg.						
72♂	174	5.46	3	55.0	61♂	5.60		346	1.888	1.084		Male-normal[3]
11♂	206	5.95	3	52.0	6♀	5.77	0.842	281	1.673	0.812	+1.02	Male-normal
31♂	225	8.13	3	53.0	25♂	4.68		210	1.807	0.813		Male-normal[3]
28♀	170	9.02	3	53.4	37♂	3.91		96	0.869	0.511		Female-normal[3]
7♀	190	9.74	3	48.0	7♀	5.29	0.450	150	1.461	0.709	+0.54	Female-normal
13♀	207	10.13	6	55.1	14♂	5.62	1.046	174	1.762	0.851	+1.54	Female-normal
10♀	226	10.88	3	51.1	21♂	4.12		127	1.385	0.601		Female-normal[3]
7♂	152	6.11	3	55.3	12♂	4.36	0.488	163	0.995	0.655	+0.67	Male-castrate 20 days
6♂	187	10.33	3	50.0	17♀	4.85	0.089	30	0.310	0.166	+0.10	Male-castrate 66 days
8♀	181	8.92	3	47.4	18♂	4.14	0.391	130	1.159	0.640	+0.50	Female-castrate 20 days
6♀	181	10.77	6	54.1	10♂	4.62	0.603	101	1.088	0.599	−0.42	Female-castrate 20 days
7♀	160	8.64	3	46.5	12♀	4.42					−0.33	Female-castrate 66 days
8♂	171	9.11	3	53.0	18♂	4.03	0.342	114	1.039	0.606	+0.36	Castrate, 200 I.U. progynon B, 20 days
9♀	160	17.40	3	47.9	18♂	3.68	0.187	62	1.079	0.674	+0.04	Castrate, 200 I.U. progynon B, 20 days
19♀	186	18.17	6	55.3	26♂	4.80	0.583	97	1.762	0.947	+0.07	Castrate, 200 I.U. progynon B, 15 days
19♀	186	18.17	12	56.7	13♂	3.72	0.204	17	0.309	0.166	+0.14	Castrate, 200 I.U. progynon B, 15 days
18♀	179	15.47	12	52.8	22♂	4.33	0.475	40	0.617	0.346	−0.26	Castrate, 200 I.U. progynon B, 20 days
15♂	190	6.26	3	53.2	24♂	4.36	0.488	162	1.015	0.534	−0.28	Castrate, 200γ oreton, 20 days
10♀	191	9.34	3	52.0	20♂	4.16	0.400	133	1.244	0.651	−0.88	Castrate, 200γ oreton, 20 days
5♂	232	11.84	3	51.6	18♂	3.81	0.245	82	0.971	0.418	+0.31	Thyroidectomized 40 days
4♂	210	11.20	3	59.2	11♂	3.80	0.240	80	0.896	0.427	+0.22	Thyroidectomized 6 months
6♀	206	13.51	3	52.6	20♂	4.16	0.400	133	1.797	0.872	+0.66	Thyroidectomized 40 days
7♂	175	6.77	3	54.55	20♂	4.26	0.444	148	1.000	0.573	+0.35	Castrate, 0.03 mg. thyroxin, 20 days
9♀	156	8.02	3	51.3	19♂	3.35	—	—	—	—	−0.29	Castrate, 0.03 mg. thyroxin, 20 days

[1] Average chick thyroid weights are total weights of paired glands.
[2] Chick units calculated from equations presented by Bergman and Turner (1939).
[3] Normal data from a previous study.

chicks were included whose normal controls had thyroids somewhat above the normal average. Further study must be given to the effect of estrogens in varying amounts upon the thyrotropin content of gonadectomized animals.

Effect of androgen. Castrate male rats weighing an average of 190 gm. were given 200γ daily of oreton (testosterone propionate) for 20 days. The pituitaries were within the normal range in weight, but contained only an average of 1.015 ch.u. in comparison to an average of 1.888 ch.u. in the normal 174 gm. rats. As the experimental rats weighed 190 gm. the average ch.u. per pituitary in the 175 and 206 gm. weight groups (ave. 190), which was 1.781 ch.u., might be the most fair comparison. On this basis there was a reduction of 43% in the hormone.

A group of ovariectomized females weighing 191 gm. were given 200γ of oreton daily for 20 days. The pituitaries were similar in weight to the normal females of the same body weight. Upon assay, they were shown to average 1.244 ch.u. per pituitary in comparison to an average of 1.369 ch.u. in the normals. This is a reduction of about 9%.

The testosterone at the injected level had very little tendency to check castration changes in the male. On the other hand, in the female, the thyrotropin content of the castrates was approximately normal.

Effect of thyroidectomy. A group of 5 male rats weighing an average of 232 gm. were thyroidectomized and held for 40 days. Their pituitaries averaged 11.84 mg. or somewhat larger than the normal controls. The assay of their pituitaries showed an average of 0.971 ch.u. each which in comparison to normals of the same weight (1.807 ch.u.) is a reduction of about 46%. In a second group thyroidectomized for 6 months there was observed a reduction to 0.896 ch.u. per pituitary or about 50%.

In a group of females thyroidectomized for 40 days there appeared to be a slight increase in pituitary weight. The assay of the pituitaries showed an average of 1.797 ch.u. of thyrotropin which in comparison to the normal female average (1.369 ch.u.) represents an increase of about 24%. Even on the basis of the comparable weight group of the normals which showed the greatest thyrotropin potency per pituitary, the thyroidectomized females at least held their own.

It is interesting that thyroidectomy should cause an apparent reduction in thyrotropin in the male pituitary whereas in the female the hormone content was maintained if not slightly increased.

Effect of castration with thyroxin. A group of male castrate rats weighing 175 gm. were injected with 0.03 mg. of thyroxin a day for 20 days. The pituitary weight, 6.77 mg., was comparable with the normal group and those castrate for 20 days. The thyrotropin content was 1.000 ch.u. per pituitary which is almost identical with the content in males castrated for 20 days. It would thus appear that the injection of thyroxin was without effect in the male castrate.

In the female castrate the injection of 0.03 mg. of thyroxin for 20 days lowered the thyrotropin content below that of the 20-day castrate since the 3 mg. level dosage failed to cause a response in the chick thyroids.

DISCUSSION

The discovery of the pituitary thyrotropic hormone, regulating in part the activity of the thyroid glands has enabled one to understand more clearly the mechanism regulating thyroxin secretion. It offers a challenge to determine in turn the factors influencing thyrotropin secretion and release. It is in this largely unexplored field, that the present study is concerned. The observation in the previous paper (1) concerning a marked rise in thyrotropin following parturition and during lactation

raised the question as to the influence of the gonad hormones. What would be the effect of gonadectomy and replacement therapy?

The present study appears to indicate quite clearly that gonadectomy of both male and female rats produces a progressive decrease in the thyrotropic content of the pituitary up to 66 days.

This work on the rat confirms the earlier observation of Reece and Turner (6) that the pituitaries of bulls from 4 to 10 months old contained 50% more hormone than steers and a 11- to 23-month old group of bulls contained 115% more thyrotropin than comparable steers.

It is of interest to note further that the injection of estrogen into the castrate female rat appeared to maintain the normal level of thyrotropin. This work should be repeated, however, due to the fact that the normal controls of the groups of chicks used in these assays had thyroid glands slightly heavier than the general average of all normal chicks.

At the level injected, the castrated male rat, however, showed the same decrease in thyrotropin as the castrated animals. It might be thought that an androgen would be more effective in the male, but our observations indicate that neither hormone, at the level administered, was capable of maintaining a normal thyrotropin content in the castrated male. On the other hand, the androgen was effective in the castrated female.

While there is much indirect evidence that the thyroid and gonads are interrelated, so far as the writers are aware, the relation of the gonad hormones to the thyrotropin content of the AP had not been previously described. It is interesting to note, in the light of these data, that Chahovitch and Vichnjitch (7) noted gradual declines in the basal metabolism following castration in a limited series of rats. Do castrated animals fatten more readily due to lowered thyroid activity as well as to reduced sex urge or is sex urge a manifestation of thyroid activity maintained by the gonad hormones?

These data further indicate the possibility of reducing excessive secretion of the thyrotropic hormone by means of gonadectomy rather than thyroidectomy in certain hyperthyroid cases. Conversely, the excessively fat condition of hypogonad individuals might respond to the gonad hormones through their effect upon the AP thyrotropic hormone.

The studies of Lebedewa (8) and Zeckwer (9) are contradictory in regard to the influence of thyroidectomy upon the thyrotropic hormone of the rat AP. In the rabbit, Chen and van Dyke (10) observed an increase in thyrotropin content only in the female rabbit 3 months after thyroidectomy. Our observations upon the rat also indicate a sex difference in that the female thyroidectomized animals showed maintenance or a slight increase in hormone content whereas the males showed a reduction. These data indicate that the rate of secretion of the thyrotropic hormone is controlled or regulated to a far less extent by the hormone of the gland which it influences (thyroxin) than is shown by the interrelation between the gonadotropic hormone and the gonad hormones (estrogen and androgen).

SUMMARY

A study is reported on the thyrotropic hormone content of the pituitaries of albino rats of both sexes weighing between 150 and 200 gm. following various periods of gonadectomy. Similar reports are given of castrate animals which were injected with estrogen (progynon-B) and androgen (oreton).

Following gonadectomy, the thyrotropin content of the AP of both male and

females was slightly reduced after 20 days and rather markedly reduced after 66 days.

Replacement therapy with estrogen at the rate of 40 R.U. and androgen at the rate of 200γ daily appeared to maintain the normal level of thyrotropin in the castrate female but not the male.

Thyroidectomy of male rats for periods of 40 days and 6 months resulted in a reduction of about 50% in the thyrotropin content of the AP. In contrast, females similarly treated maintained their normal content or showed a slightly increased level of hormone.

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THE EFFECTS OF OVARIECTOMY AND ADMINISTRATION OF PROGESTERONE ON THE ADRENAL X-ZONE AND THE UTERUS

EVELYN HOWARD AND SAIYUDE GENGRADOM
From the Department of Physiology, The Johns Hopkins University School of Medicine
BALTIMORE, MARYLAND

THE FIRST PREGNANCY in a young adult mouse has a very marked effect on the adrenal cortex. The X-zone, comprising nearly half of the cortical tissue in young non-bred female mice, disappears in most instances by the fourteenth day of pregnancy (1). This effect of pregnancy on the adrenal cortex is of sufficient interest to call for further analysis. It has been shown that in males the X-zone is promptly responsive to testis hormone; it hypertrophies following castration (1) and this hypertrophy is inhibited by androgens (2). In addition, the presence of a well developed X-zone tends to be associated with a stimulation of the seminal vesicle epithelium in castrates (3, 4). These relationships suggest that the X-zone may produce androgens, and in sufficient quantity to influence the seminal vesicles, although other interpretations are by no means excluded by present evidence.

In attempting to arrive at an understanding of the significance of the X-zone, it is of interest to know whether the removal of the X-zone by male hormones is a specific reaction or whether the adrenal reaction may be evoked by a sufficiently high concentration of ovarian hormones. The fact that the reaction is typical of a first pregnancy in mice need not lead one to expect that this too is not a reaction to androgens, since various workers have reported the presence of androgens in the urine of pregnancy, and Marker et al. (5) have isolated an androgen, epi-allo-pregnanolone, from pregnancy urine.

There is little evidence that the X-zone exerts any specific effect in female mice (6, 7). Nevertheless, since this zone is normally much more developed in female than in male mice, its function in the former requires consideration. If females are not bred, the zone gradually degenerates. Thus the effect of pregnancy is to hasten a normal process. A gradual degeneration occurs also in ovariectomized females, so that hormones produced by the ovary are not essential for the process.

The Effect of Androgen on the X-Zone in Females

Other workers (2, 8) have shown that androgens exert a prompt effect on the X-zone both in castrated males and in females. We have made further observations on the effect of testosterone on the X-zone in females. Two mice were ovariectomized at 3 weeks of age, and at 5 weeks were given daily injections of 0.2 mg. testosterone propionate in oil, for a period of 15 days. Two controls were ovariectomized and injected with oil. The animals were killed at 54 days of age. Large X-zones were present in the controls, while in those treated with testosterone the X-zone had disappeared except for small amounts of fibrous tissue. Thus we can confirm previous findings that the X-zone in females is inhibited by testis hormone.

The Effect of Ovariectomy on the X-Zone

If the ovary is the source of hormones which cause degeneration of the X-zone during pregnancy, it seemed possible that repeated ovulatory cycles might exert some influence in hastening the X-zone degeneration in non-bred females. Previous observations by one of us (1) indicated that during periods up to one month following ovariectomy in adults there was exhibited no appreciable effect of the ovarian deficiency on the progress of the X-zone degeneration. However, it seemed desirable to supplement these earlier observations by a study of the effects of ovariectomy performed on immature mice and followed throughout the whole period of X-zone development and degeneration. Consequently we have followed the process in a group of females, 30 ovariectomized at 20 days of age and 30 normal controls, in most cases litter mates. The animals were killed after intervals of 20, 40, 60, 100, 140, 180 and 200 days. The adrenals showed some evidence of an effect of the ovaries, in that groups aged 40, 60 and 80 days had larger X-zones (about 30% greater in width) after ovariectomy than did the corresponding normal groups. Both ovariectomized and normal animals aged 120 days and over showed marked degeneration of the X-zone. In the ovariectomized group there were a few exceptions which exhibited a condition indicative of some tendency for the X-zone to persist or redifferentiate. Since the number of animals in any one group was not very great, and since considerable individual variation was encountered in the older groups, much weight cannot be given to these cases of prolonged persistence of the X-zone in the ovariectomized animals. However, the data indicate that the ovary may have some inhibitory effect on X-zone development, but that it is of a minor degree compared to the effect of a pregnancy.

Some observations on the effect of estrogens on the X-zone have been made which indicate that estrogens tend to stimulate X-zone growth. Thus Martin (9), Burrows (10) and Starkey (11) all agree that estrogen injections cause a persistence and reappearance of the X-zone in males. Their evidence regarding the effect of estrogens in females is less clear-cut. Starkey found that estrin administered over short periods of time diminishes the degenerative process in the X-zone of adolescent females, while Martin found that prolonged treatment with estrogen is not incompatible with the occurrence of the degenerative process. However, so far as the evidence goes, most of it indicates that the effect of estrogen injections on the X-zone tends to be somewhat opposite to that of androgens.

The Effects of Progesterone Injections on the Uterus and on the X-Zone[1]

We have attempted to see whether or not the effects of pregnancy on the adrenal could be duplicated by giving progesterone. Preliminary experiments with injections of progesterone in oil of the same order of magnitude as were effective with testosterone were without effect in causing degeneration of the X-zone in young ovariectomized females. Hoping to obtain a greater effective continuity of treatment, we then used pellets of progesterone in a group of 5 ovariectomized, month-old mice. These animals carried pellets of progesterone subcutaneously for 4 weeks, during which period solution of progesterone in the body fluids occurred at the rate of 0.137 mg. per mouse per day. At the end of this period the adrenals all contained large well developed X-zones without relevant differences from those in the litter-mate controls. The uteri of the treated animals were 30 to 40% larger in diameter than

[1] We are indebted to the kindness of Dr. E. Oppenheimer, of Ciba Pharmaceutical Products Inc., for the crystalline progesterone used in these experiments. Dr. Oppenheimer stated that Ciba had ascertained the absence of estrogenic substances in this progesterone.

those of the controls, and the lining epithelium showed qualitative evidence of stimulation in that the cells were more columnar. There was also some evidence of increased proliferation of the endometrium.

This progesterone treatment of spayed mice was then repeated in intact females to see whether or not the amount of estrogens or other substances produced by the ovaries in the presence of progesterone pellets would be effective in reproducing the effect of pregnancy on the X-zone. Progesterone pellets were implanted in 6 females at 33 days of age, and the animals killed, after a period of 21 days, together with an equal number of litter-mate controls. The adrenals all contained large well developed X-zones, which showed no appreciable differences as a result of the progesterone treatment.

Robson (12) has evidence that in pregnancy in mice a concentration of progesterone is present similar to that produced by the daily administration of 1.5 mg. of the luteal hormone. On the basis of the work of Deanesly and Parkes (13) on the comparative efficacy of injections and pellet treatments with androgens, one might expect that the treatment with progesterone pellets would have been more effective physiologically than the intraperitoneal injection of 1.5 mg. daily. However, there is some evidence that pellet treatments do not give duplications of physiological maxima under all conditions. Accordingly we made observations on the effects of injections of larger amounts of progesterone than were dissolved from pellets.

Injections, intraperitoneally, of 1.5 mg. in oil daily for 14 days in 2 ovariectomized mice produced in one an X-zone smaller than that usual at this age. However, the other mouse was not appreciably affected. In order to give as high a continuous dosage of progesterone as seemed physiologically practicable, mice received subcutaneous pellets of progesterone and in addition daily injections of approximately 5.5 mg. of progesterone in oil, intraperitoneally. (400 mg. of progesterone were dissolved in 10 cc. of corn oil. A little (ca. 10 to 20 mg.) progesterone crystallized out of the oil during the injections. Thus each mouse received in 14 days 2 cc. of oil containing a saturated solution of progesterone.) The animals had been ovariectomized at 3 weeks of age, and were treated for a period of 14 days beginning at 30 days of age; they were killed at 47 days of age. Litter-mate controls were ovariectomized at the same age and injected with equivalent quantities of oil. A considerable amount of unabsorbed oil was present at autopsy (as well as residues of the pellets) although 3 days had elapsed since the termination of the oil injections. Thus it is impossible to be certain how much hormone was absorbed, but presumably these mice were treated with more hormone than they were able to absorb, and hence received as large a dose as it is possible to give by a combination of pellets and oil injections. In evaluating the dosage used, it may be noted that 1 mg. daily gives a marked uterine response in monkeys (14), 2 to 5 mg. daily maintains adrenalectomized ferrets and rats (15, 16) while 3 mg. daily has androgenic effects on the prostate and the clitoris in immature rats (17).

Body weights were less in the group which had received progesterone (ave. 16.9) than in the controls (ave. 18.9). The mice treated with progesterone had well developed uteri in marked contrast to the small uteri of the controls. The uterine diameter had increased 300 to 400%, with a generalized growth of all layers. There was an increase in endometrial complexity greater than that described by Deanesly and Parkes (18) as that reached during the 11th day of pregnancy, but somewhat less than the maximum typical of pregnancy, as found in the sterile horn of a uterus on the 17th day of pregnancy. Thus the progesterone dosage used was sufficient, even in the absence of preliminary estrogen treatment, to give a uterine reaction not greatly

inferior to the normal reaction to pregnancy. These findings of uterine stimulation by progesterone alone in mice are in accordance with results obtained by Hisaw, Greep and Fevold (19) in monkeys and rabbits, and to a lesser degree in rats. In view of the isolation of estrone from the adrenal (20) and physiological evidence suggestive of gynecogenic activity by the adrenal (21) it may be asked whether estrogens from the adrenals are not available to give some degree of synergistic action with progesterone in this type of experiment.

The adrenals of the controls had very large well developed X-zones showing beginning vacuolation. In the animals which received progesterone the X-zones were somewhat smaller, both absolutely and in relation to total cortex, than in the ovariectomized controls. In 3 out of the 5 animals their size was essentially the same as in a normal female of this age. In 2 mice there was somewhat greater reduction in the proportion of the X-zone, but in these cases there had been definite retardation of body growth (12.8 and 16.4 gm. respectively, in comparison with 18.9 gm., ave. of controls). Hence the adrenals in these two cases were probably affected by factors involved in a systemic growth failure, which may have been a result of a somewhat generalized pituitary inhibition. In any case it may be seen that progesterone treatment does not cause the complete removal of the X-zone which occurs after treatment with much smaller quantities of testosterone propionate.

These findings indicate that the X-zone is much more sensitive to substances of a more specific androgenic nature than it is to progesterone, although there may be some tendency of progesterone to affect the adrenal in a similar way.

DISCUSSION

Since the reaction of the mouse adrenal to pregnancy has not been imitated by treatment with estrogen or progesterone, whereas it is by testosterone and other androgens, this adrenal reaction seems to furnish an indicator of some considerable increase in the physiologically effective level of androgens during the first pregnancy in mice. In this connection it is interesting to note that Turner (22) has found, in studying sperm development in testicular grafts, that pregnant females furnished a considerably more favorable environment for sperm maintenance than did non-bred females. The tendency for an increase in maleness of the female during pregnancy may perhaps not seem anomalous if the pregnant organism is considered as an environment for growing tissues of both sexes.

However, in addition to any physiologically effective increase in androgens which may occur during pregnancy, other factors are by no means excluded as having a bearing on the histological changes in the cortex of the young adult mouse during a first pregnancy.

SUMMARY

Ovariectomy has only a slight stimulating effect on the growth of the adrenal X-zone. Pregnancy is associated with a rapid degeneration and disappearance of the X-zone, and the same effect is produced by the injection of androgens. However, progesterone treatment, up to 5 mg. daily, does not produce any marked degeneration of the X-zone, and hence does not reproduce the effect of pregnancy on the adrenal. Progesterone can produce marked stimulation of uterine growth in ovariectomized mice, without preliminary treatment with estrogens.

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THYROTROPIC HORMONE AND FLUORINE ACTIVITY

FLOYD DEEDS, ROBERT H. WILSON AND WINDSOR C. CUTTING

From the Bureau of Agricultural Chemistry and Engineering,[1] *United States Department of Agriculture, and the Department of Pharmacology, Stanford University School of Medicine*

SAN FRANCISCO, CALIFORNIA

IN AN EARLIER PAPER Wilson and DeEds (1) called attention to the synergistic action of orally administered thyroid on the production of bleached rat incisors by fluorine, and briefly reviewed the claims of other authors relative to a relationship between the thyroid gland and fluorine toxicity. At that time the observations of Wilson and DeEds were correlated with those of Karnofsky and Cronkite (2) on the effect of thyroxin on eruption of teeth in newborn rats. The present paper deals with the relationship of the anterior pituitary to fluorine toxicity as manifested by an increased susceptibility to the bleaching action of ingested fluorine on rat incisors produced by the daily injection of thyrotropic hormone.

Earlier observations on the relationship of the pituitary gland to fluorine toxicity have been made by Cristiani (3) and by Phillips, Lamb and coworkers (4, 5, 6). Cristiani reported macroscopic changes in the hypophysis of guinea pigs showing chronic fluorine poisoning, claiming that a diminution in volume of the gland occurred, and that it became flat in appearance. On the other hand, Phillips et al., using rats, reported that the weight of the hypophysis, the microscopic picture, and the gonad-stimulating function remained normal.

In summarizing their observations Karnofsky and Cronkite (2) stated: "The rate of the incisor teeth eruption in newborn rats is markedly accelerated by thyroxine; time of eruption varying with the dosage used." Their attempt to use this phenomenon as a method for bioassay of the thyrotropic hormone was unsuccessful. This failure might be due to insufficient delicacy in the criterion of action, or to the fact that the rat is unusually refractory to the thyrotropic hormone. The use of a species known to be refractory would in itself necessitate the use of a delicate criterion in the development of a bioassay method for thyrotropic hormone.

The known delicacy of the response of the albino rat to the toxic action of fluorine, and the previously reported marked synergistic action of thyroid on the production of bleached rat incisors by fluorine suggested that incisor tooth coloration in albino rats maintained on a diet containing minimal amounts of fluorine might be used as a basis for the bioassay of thyrotropic hormone. Even if a practical bioassay method did not prove feasible, the problem appeared attractive from two other points of view. The experiment might demonstrate the existence of a response to thyrotropic hormone in a refractory species, and secondly, it might yield additional interesting information regarding glandular relationships in fluorine poisoning.

The experimental procedure was as follows. Twenty female albino rats averaging 50 gm. in weight were divided into 4 equal groups and placed on the basic diet previously described (1). This diet contained 0.001% of added fluorine. Group 1 served as controls, and groups 2, 3 and 4 received daily, except Sunday, subcutaneous injections of 0.02, 0.1 and 0.5 cc. of thyrotropic extract respectively. The thyrotropic extract was prepared from beef anterior pituitary glands by the flavianate method (7).

[1] Food Research Division Contribution No. 476.

The rate of incisor tooth coloration was evaluated and plotted as previously described (1). The results of the first experiment are shown in figure 1.

Inspection of the graph shows that the characteristic coloration of the white incisor teeth of the control rats proceeded rapidly and was complete by the 23rd day. The lowest dosage of thyrotropic hormone also permitted rapid coloration of the teeth although the rate was perhaps somewhat slower than in the control group, not being equal to that of the controls until about the 27th day. The rats receiving 0.1 cc. of thyrotropic hormone showed a practically normal rate of tooth coloration until the 10th day, but from the 10th to the 17th day the rate of coloration was definitely re-

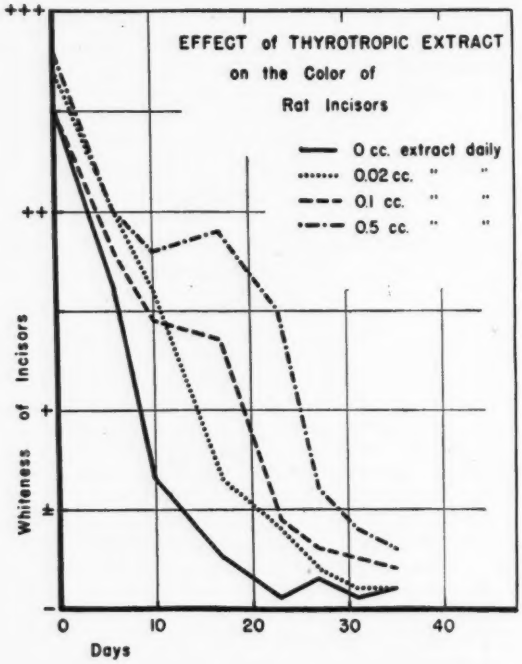

Fig. 1

tarded. From that time until the end of the experiment a more rapid and practically normal rate of coloration took place. On the highest dosage, 0.5 cc. of thyrotropic hormone, the rate of tooth coloration started to deviate sharply from normal on the 10th day and actually showed increased bleaching until the 17th day, after which time the development of incisor tooth coloration was resumed. The failure to retain bleached teeth after the 17th day on the two highest dosages of thyrotropic hormone probably is due to the development of a refractory state, evidence for which is found in the measurements of metabolic rate.

Since the rat is known to be rather refractory to the action of thyrotropic hormone, it did not seem necessary to measure metabolic rates except on the controls and those receiving the highest dosage of the hormone, i.e., 0.5 cc. Metabolic rate determinations were made on the 18th, 24th and 32nd days of the experiment. On these days the oxygen consumptions of the rats receiving thyrotropic hormone were,

respectively, 0.17, 0.13 and 0.06 cc. of oxygen per 100 sq. cm. of body surface per minute higher than those of the controls, which gave values of 1.90, 1.87 and 1.88 cc., respectively. These differences in oxygen consumption paralleled the effect noted on the coloration of the incisors. The metabolic rate determinations were made with a modification of the Tainter-Rytand (8) apparatus.

The experiment was repeated with 10 rats averaging 50 gm. in weight, 5 animals serving as controls and 5 receiving 1.0 cc. of the thyrotropic hormone, or double the

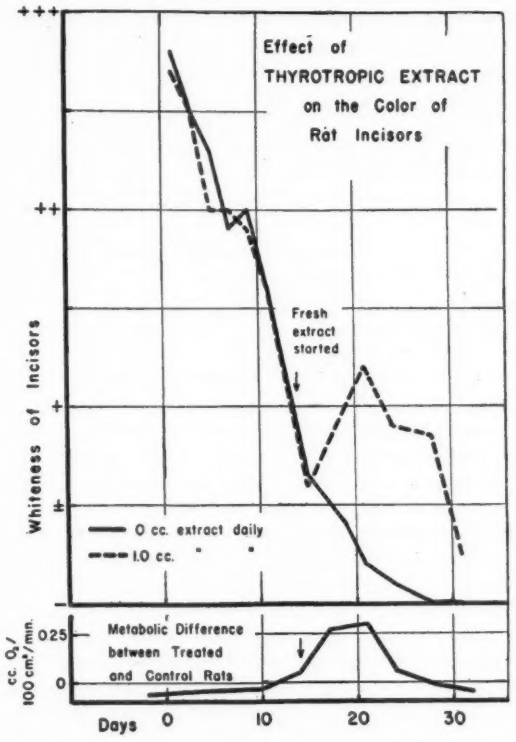

Fig. 2

highest dosage used in the preceding experiment 2 months earlier. The extract was the same preparation used previously, and had been kept in a refrigerator. On the basis of the first experiment and as the result of experience gained on the studies dealing with the synergistic action of thyroid on fluorine toxicity (1) a differentiation between the control rats and those receiving the hormone should have appeared about the 10th day of the experiment. However, no difference in coloration of the incisor teeth was observed even as late as the 15th day. Metabolic rate determinations were made on all rats 2 days before the start of thyrotropic hormone injections, and again on the 8th, 10th and 14th days following the start of the injections. The differences in metabolic rate between the average for the experimental rats and the controls were respectively −0.06, −0.03, −0.03 and +0.05 cc. of oxygen per 100 sq. cm. of body surface per minute. The failure of the thyrotropic hormone to produce either a bleach-

ing of the incisor teeth or elevation of the metabolic rate suggested that the anterior pituitary extract had deteriorated during the 2 months of storage in the refrigerator. Accordingly injections of a freshly prepared extract were started on the 14th day.

On the 19th day, i.e., the 5th day of the new regime, the incisor teeth of the rats receiving thyroptropic hormone were distinctly whiter than those of the controls. This differentiation between the two groups increased and was maximal on the 21st day, the 7th day of the new regime. Meanwhile the increase in coloration of the incisors of the control rats progressed normally. From the 21st day to the close of the experiment the bleaching observed on the teeth of the rats receiving the fresh extract diminished, but coloration remained somewhat less than that of the controls. Parallel to these changes in tooth coloration were the changes in metabolic rate. Injections of the fresh extract were started on the 14th day of the experiment and metabolic rate determinations were made on the 17th, 21st, 24th, 28th and 32nd days. On these days the differences in metabolic rate between the average for the experimental rats and the controls were respectively +0.27, +0.30, +0.06, −0.01 and −0.05 cc. of oxygen per 100 sq. cm. of body surface per minute. The results of this experiment are shown in figure 2.

The results obtained in these two experiments are in agreement. The negative response to thyrotropic hormone in the first part of the second experiment, both in regard to tooth coloration and metabolic rate, probably was due to the use of an old extract as evidenced by a positive response when a fresh preparation was employed. In fact, the evidence in the second experiment is more striking than that of the first. In the first experiment effective dosages of thryotropic hormone retarded the development of tooth coloration until the rats became refractory to the action of the extract. During the time that old extract was employed in the second experiment tooth coloration progressed normally in both the experimental and control groups, but after injections of the fresh extract were started tooth coloration not only stopped, but actual bleaching occurred until the rats became refractory.

It is concluded from these experiments that young albino rats placed on a diet containing a known but small amount of added fluorine respond to the action of thyrotropic hormone by showing either a retarded rate of coloration of the incisor teeth or an actual bleaching. The effect on tooth color is a more delicate criterion of thyrotropic hormone action than is the time of tooth eruption. The effect of the hormone on tooth color is believed to be mediated through the stimulation of the thyroid gland. Since the action of thyrotropic hormone as observed on tooth color occurs within 10 to 15 days the procedure described in this paper offers an interesting possibility for the development of a bioassay method for thyrotropic hormone. The possibility of increasing the sensitivity for bioassay purposes by determining the optimum concentration of added fluorine is worthy of further investigation.

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THE EFFECTS OF ANTUITRIN-S,[1] PROGESTIN AND A VITAL DYE ON LATE PREGNANCY IN THE RAT[2]

RUSSELL M. COCO

From the Zoological Laboratories, Louisiana State University
UNIVERSITY, LOUISIANA

A NUMBER OF INVESTIGATORS have studied the effects of pregnancy urine and corpus luteum substances on pregnancy. Prolonged gestation with post-mature fetuses in rabbits and rats by the use of P U extract (antuitrin-S) injected in late pregnancy has been reported (1–5). Crude P U extract gave results similar to those obtained with antuitrin-S (6, 7, 8).

Extended pregnancy in the rat and ground squirrel by the injection of crude corpus luteum extracts has been reported (9, 10, 11). However, Bunde (12) was unable to prolong gestation in the rat with large doses of highly purified and active progestin. Allen and Heckel (13), by using progesterone, failed to prevent resorption of embryos in rabbits castrated in very early pregnancy, but in a later paper (14) they were able to maintain pregnancy in rabbits castrated on the 11th day of gestation.

It is the consensus of opinion among investigators in this field that it is the secretion of the corpora lutea administered in the form of an extract or produced by the induced corpora lutea in the animal's own ovaries which is responsible for the prolongation of pregnancy.

MATERIALS AND METHODS

Except for a few animals of the hooded variety, the rats used in these experiments were of albino stock, *Mus norvegicus albinus* Hatai 1907. A total of 170 animals was used. The gestation period for the colony as a whole and for 4 animals injected with Locke's solution was found to be 21 to 22 days. Males were placed in the mating cages at night and vaginal lavages were made the following morning; the day that spermatozoa were found was taken as the first day of gestation. The presence of the placental sign (15) was observed and recorded.

Injections were usually made in the forenoon. After being injected, each animal was kept in an individual cage and was regularly examined twice daily (morning and night), and in special cases constant or hourly observations were made. When an exploratory laparotomy or autopsy was performed, the condition of the reproductive tract was carefully examined and recorded.

The antuitrin-S used in this investigation was standardized to contain not less than 100 R.U. per cc. Several vials of the extracts were reassayed on rats in our colony according to Kamm's method, but no significant variations from his assay were obtained.

RESULTS AND DISCUSSION

It seems advisable at this point to redefine the terms 'prolonged gestation' and

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'postponed parturition,' since they are rather loosely used in the literature. In this paper the term, prolonged gestation, is used to indicate that condition which exists when a pregnant animal goes past term with the fetuses still alive and growing in utero. The term, postponed parturition, is used to indicate that condition which exists when the fetuses die in utero before or at term but due to the failure of the birth mechanism and weakness of the mother, or to some other reason, the female is unable to give birth to her young.

Effect on parturition and condition of the fetuses. Seventy-one animals were injected with antuitrin-S in single doses of 10 to 200 R.U. ranging from the 14th to

TABLE 1. RESULTS OF INJECTING ANTUITRIN-S INTO PREGNANT ANIMALS

No. of animals	Amount inj.	Day of pregnancy inj.	Subsequent history[1]	No. of animals	Amount inj.	Day of pregnancy inj.	Subsequent history[1]
	R.U.				R.U.		
2	50	18	1-d.f.-20; 1-l.f.-22.	2	150	18	1-d. & l.f.-21; 1-d.f.-23.
1	65	18	1-d.f.-22.	2	200	18	1-d.f.-24, 25, 26, 27; 1-d. & l.f.-25.
1	70	18	1-d.f.22.	1	200	19	1-l.f.-22.
9	70	18	2-d.f.-21; 3-d. & l.f.-21; 1-d.f.-21; 2-d.f.-22; 1-d. & l.f.-22.	2	30 daily	15–16 inc.	1-d. & l.f.-19; 1-l.f.-19.
1	75	15	1-d. & l.f.-22.	3	25 daily	15–18 inc.	2-d.f.-21; 1-d.f.-20.
4	75	16	2-d.f.-20; 1-d. & l.f.-20; 1-d. & l.f.-21.	1	10 daily	14–21 inc.	1-l.f.-22.
4	75	17	2-d.f.-21; 1-l.f.-18; 1-l.f.-19.	1	10 daily	16–21 inc.[a]	1-l.f.-22.
14	75	18	1-l.f.-19; 3-d.f.-20; 6-d.f.-21; 1-l.f.-21; 1-d. & l.f.-21, d.f.-22; 1-l.f.-22; 1-d. & l.f.-22.	1	15 daily	15–17 inc.	1-d.f.-18.
6	75	19	2-d.f.-22; 1-l.f.-22; 3-d. & l.f.-22.	1	15 daily	14–21 inc.	1-l.f.-22.
3	75	20	3-l.f.-22.	1	15 daily	14–19 inc.	1-d.f.-20.
1	75	21	1-l.f.-23.	2	25 daily	17–21 inc.	2-l.f.-22.
5	100	18	2-d.f.-20; 1-d.f.-21; 1-d.f.-21, 22, 23, 24, 25, 26; 1-d.f.-24.	1	25 daily	17–20 inc.	1-d.f.-20.
2	150	16	1-d.f.-21; 1-d.f.-24.				

[1] Condition of fetuses was ascertained by exploratory laparotomies, autopsies, or by the occurrence of birth. Abbreviations used are as follows: d.f., dead fetuses; l.f., living fetuses; d. & l.f., dead and living fetuses; R.U., rat unit; inc., inclusive; inj., injected. 2-d.f.-22 means 2 animals gave birth to dead fetuses on the 22nd day; 1-d.f.-21, 22, 23, 24, 25, 26 means 1 animal gave birth to dead fetuses on the 21st, 22nd, 23rd, 24th, 25th and 26th days. [a]17th day excepted.

the 21st day of gestation. It is evident from table 1 that animals injected on the 15th to the 20th day with dosages of 50 to 75 R.U. gave birth to dead, living, or dead and living fetuses before or at term (fig. 1). Young born alive were usually not viable. In no case within this series was gestation prolonged, nor was postponed or prolonged parturition detected even after hourly and in several cases continuous observations. In this connection it is important to note that dosages similar to those employed by Hoopes (3) and Hoopes and King (4) did not prolong gestation nor did they postpone or prolong parturition, and in no case were fetuses found living in utero past term as they described. Dosages of 100 R.U. administered on the 18th day of pregnancy gave varying results as follows: 1 case of premature birth, 1 of birth at term, and 2 cases of postponed and prolonged parturition. In all cases the fetuses were dead at parturition, and in many instances they appeared compressed. When dosages of 150 to 200 R.U. were used, 4 out of 7 animals showed parturition to be

postponed and prolonged for as long as 1 to 5 days. With one possible exception, these were definitely not cases of prolonged gestation since the fetuses died before or at term. In this series of animals necrosis and autolysis of the fetuses had proceeded so far that an exceedingly offensive odor was nearly always present after the animals had gone beyond term. The mothers often died as a result of this postponed parturition.

It is believed that the induced corpora lutea were not responsible for these cases of postponed parturition since the fetuses died before or at term (one exception cited above), and some animals with well-formed, induced corpora lutea gave birth prematurely or at term to dead or non-viable fetuses. Furthermore, as shown by subsequent histories, 2 animals copulated and became pregnant on the same day that abortion occurred, 2 others conceived on the 3rd day and 1 on the 7th day postpartum. Also dianil blue alone postponed and prolonged parturition in some animals, and progestin failed to prolong gestation.

Data obtained on the condition and size of the fetuses of 71 animals injected with 10 to 200 R.U. of antuitrin-S on the 14th to 21st day (table 1), showed that of a total of 566 fetuses accounted for by the presence of placental sites (which indicated that the mothers had eaten their young), by exploratory laparotomy, autopsy or birth, only 105 were living 72 hours postpartum, the remaining 461 being, of course, non-viable. Thus 18.6% of the fetuses were viable and 81.4% were non-viable. Of 270 fetuses from 32 animals injected on the 18th day with dosages of 50 to 200 R.U., 97.6% of these were non-viable and 2.4% viable. Day 18 is important since it corresponds more or less to day 19 of Hoopes and King's experiments.

Further proof that antuitrin-S resulted in injury to the fetuses is borne out by the fact that some of those born alive were placed with foster mothers which had young of comparable ages, but they failed to survive. In cases in which experimental animals had viable young, these were usually not up to par physically as shown by their growth curves. Young taken from uninjected mothers and placed with injected mothers immediately after they had given birth did not survive. This would indicate impaired lactation.

Condition of the uteri. On the second day after injection, a yellowish, mucoidal fluid was usually noticed oozing from the vagina. As pregnancy proceeded the color of the secretion changed to a reddish hue, and later to a dark red color, due to the leakage of blood from the placentae which were losing their uterine connections, and later to the autolysis of necrotic fetuses. The exudation of blood from the vagina could often be caused by gently handling the animal. At autopsy an appreciable quantity of dark-colored blood was usually found to be present in the uterus. The source of this blood was traced to the placentae which were losing their uterine attachments.

Histological examinations of the uteri indicated that in a few cases a partial sloughing off of the epithelia had occurred but this was by no means the rule. That the injury was not of a permanent nature was indicated by the fact that 2 females receiving 75 R.U. each on the 18th aborted on the 20th and 21st days, respectively, and immediately became pregnant. Three others became pregnant within 3 to 7 days postpartum.

Condition of the ovaries. It is important to correlate the histological changes in the ovaries with the physiological phenomena of abortion, parturition, and prolonged gestation. With this in view a histological study of the ovaries of 24 injected animals was made and compared to those of uninjected pregnant animals.

In order to positively identify the corpora lutea of pregnancy from those induced

by antuitrin-S, a vital dye, dianil blue 2R, was used in 15 animals. This dye enables one to distinguish positively the corpora lutea of pregnancy from those induced by antuitrin-S injections, since those of pregnancy take up the dye while the induced ones (those formed after dye injection) do not. Thus the ovary usually showed blue and flesh-colored, unstained corpora in animals receiving dianil blue when it was removed 48 hours or longer after the injection of antuitrin-S. A second criterion used to distinguish these corpora was the size and number of the lipoid granules when Benda's fluid had been used as a fixative. The corpora lutea of pregnancy contained larger but fewer granules while the induced ones contained smaller but more numerous ones. The size and number of the granules can be used as a measure of the amount of regression that has taken place (15).

The usual procedure for injection was as follows. On each of 4 consecutive days beginning with the day the placental sign appeared (usually the 14th day of pregnancy), the animals were injected intraperitoneally with 3 or 4 cc. of a 1% aqueous solution of the dye, making a total of 12 to 16 cc.

Antuitrin-S was administered subcutaneously generally the day following the last injection of dianil blue; the condition of the animal was always carefully checked and recorded before injecting the hormone. The ovaries were also carefully examined when an exploratory laparotomy or autopsy was performed. Ovaries were removed from 24 animals at intervals of 12, 24, 36, 48, 60, 72, 84, 96 and 120 hours after injection of the hormone in order to determine the time required for various dosages injected on different days to cause luteinization, and to correlate these changes with the condition of the fetuses.

From these studies, it may be stated that in general the injection of 75 to 100 R.U. of antuitrin-S on the 18th day of pregnancy resulted in the luteinization of mature follicles within 3 to 5 days but this luteal tissue did not become functional enough to prolong gestation (fig. 2). Not uncommonly it was found that large luteal cysts were present (fig. 3); the antra of some of these were often filled with blood (fig. 4). Some ovaries contained exceedingly large follicles, but these were for the most part cystic and atretic; when they were present, they were usually the predominant structures in the ovary. No significant difference in the results obtained with antuitrin-S could be detected between primiparous and multiparous animals.

Ovulation was not found to occur during pregnancy, but followed its termination. At no time were ova detected in the oviducts. In 4 cases of injected females ova were seen trapped in the newly formed corpora lutea, indicating that ovulation had not taken place.

It is the author's belief that in most cases these induced corpora lutea did not function to any appreciable extent, if in some cases indeed they functioned at all. Support for this opinion is found in the following facts: gestation was not prolonged (1 possible exception out of 71 cases) and in some cases birth was premature even though well-formed induced corpora lutea with small antra were present in the ovaries; 2 females were seen in copulation and became pregnant on the same day they aborted, and 2 additional animals copulated and became pregnant on the 3rd day postpartum; progestin in doses as large as 5 RB.U. did not prolong gestation as shown in these experiments and those of Bunde (12).

Condition of the placentae. Regardless of the dosage or day of injection, in no case were fetuses born or found dead in utero previous to the latter half of the 2nd day following the administration of antuitrin-S. However, dead fetuses were found in the last half of the 2nd day postinjection, and the placentae were not found to be appreciably loosened from their uterine attachments previous to the 2nd day. The pla-

centae first lost their attachments along the periphery as was indicated by the fact that not uncommonly a whitish, anemic ring was present along the outer edges. In many cases when they were pulled loose with a pair of forceps, it could be seen that the central parts of the attachment surfaces, if such were still present, were still intact and vascular while the outer parts had lost their vascularity. Histological

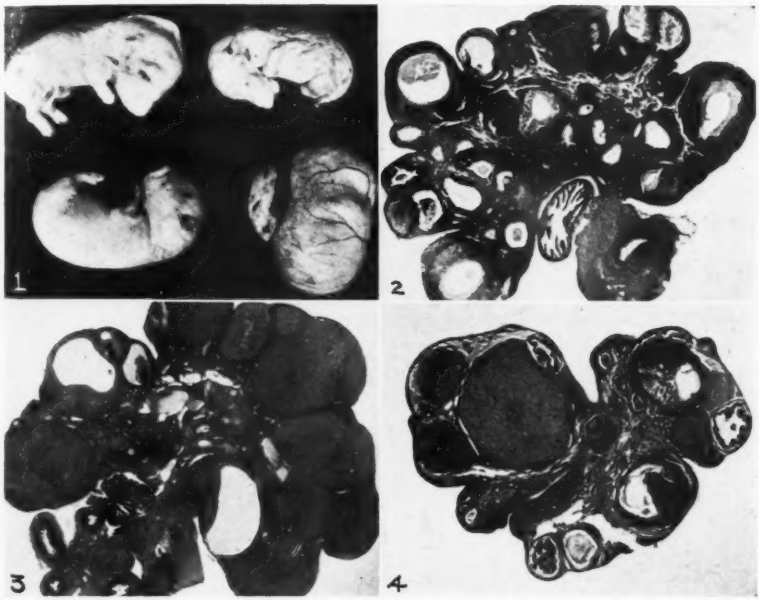

Fig. 1. THREE DEAD AND 1 LIVING FETUS BORN PREMATURELY ON THE 20TH DAY TO ANIMAL 38003(d) WHICH HAD RECEIVED 75 R.U. OF ANTUITRIN-S ON THE 16TH DAY OF GESTATION. ×1.2. The 3 dead young weighed 1.7, 1.6, and 0.9 gm., respectively. The 4th young was still living within its fetal membranes, and it together with the membranes and placenta weighed 2.3 gm. The fetuses were preserved and photographed in 95% alcohol. Fig. 2. A SECTION THROUGH THE RIGHT OVARY OF ANIMAL 38008(h) WHICH HAD RECEIVED 25 R.U. OF ANTUITRIN-S ON EACH OF DAYS 15, 16, 17 AND 18. ×11. She was killed on the 19th day. Autopsy revealed one dead fetus in utero. The ovaries contained hemorrhagic follicles, cystic and atretic follicles, induced corpora and corpora of pregnancy. Fig. 3. A SECTION THROUGH THE LEFT OVARY OF ANIMAL 38008(d) WHICH HAD RECEIVED 100 R.U. OF ANTUITRIN-S ON THE 18TH DAY OF GESTATION. ×11. She was killed on the 26th day since she was in a poor physical state and had not yet given birth to her young. Autopsy revealed 10 dead fetuses in utero. The only induced luteal tissue was that represented by the 2 large luteal cysts shown with large antra. The right ovary contained 2 hemorrhagic follicles. This animal represents a typical case of postponed parturition. Fig. 4. A SECTION THROUGH THE RIGHT OVARY OF ANIMAL 38007(c) WHICH HAD RECEIVED 150 R.U. OF ANTUITRIN-S ON THE 18TH DAY OF GESTATION. ×11. She was killed on the 20th day as she was not doing well and had been bleeding from the vagina since the 19th day. Autopsy revealed 4 dead and 3 living fetuses of normal size. Note the 3 atretic follicles with convoluted walls, 1 hemorrhagic follicle and 1 hemorrhagic, luteal cyst in which luteal tissue is invading the antrum at one side.

comparisons of placentae from injected and non-injected animals were made. Placentae of the injected animals revealed vasodilation, hemostasis, infarct formation and necrosis; this was especially true in the cases of higher dosages. The maternal side of the fetal placenta was more affected. This apparently was sufficient to decrease the blood supply to the fetuses to a sufficient degree to stunt their growth, and in the majority of cases to cause their death before or at term. Further support is afforded

by the fact that some fetuses which were smaller than normal fetuses of that age were born alive prematurely. Dead fetuses found in utero were always associated with detached placentae.

The effect of progestin on pregnancy. If the induced corpora lutea were responsible for the prolongation of pregnancy in the experiments of Hoopes (3), Hoopes and King (4) and Bunde (12), then it seems logical that progestin should accomplish the same results. Animals injected with 0.8 to 5 I.U. of progestin (lipo-lutin), at the time that the induced corpora lutea were supposedly functional, gave birth to living fetuses at term. Bunde (12) was unable to prolong gestation in late pregnancy in rats by the use of highly purified and active progestin in doses as large as 3 RB.U. a day. The cases reported here substantiate the findings of Bunde.

The effect of dianil blue on pregnancy. Of 20 pregnant animals injected with dianil blue 2R in quantities of 4 to 5 cc., as used by Long and Evans (15), to test the toxicity of the dye, only 4 successfully completed their pregnancies. The dye was considered to be toxic when a dark, blood-stained, mucoidal secretion was found on the vulva since this was known to indicate that autolytic processes were taking place. It is concluded that dianil blue, in the amounts injected, is capable of producing abortion, death of the fetuses, postponed parturition, and in some cases death of the mother. Toxicity was also shown by the injection of dye in non-pregnant animals. These results are not in accord with those of Long and Evans who could detect no serious toxic effects of the dye, when it was injected into pregnant rats at any time during their reproductive histories, but they are in agreement with those of Meyer (16) who was able to inhibit the estrous cycles of rats with dianil blue. It is believed that the dye so disturbs the physiology of the animal that her birth mechanism is impaired and she is unable to give birth normally. Indeed in some cases the dye was so toxic that the mother often died.

It is difficult to reconcile the results of these experiments with those of Hoopes (3) and Hoopes and King (4). It is possible that the antuitrin-S used by them was of a different potency than that used here. However, since they do not state whether or not they reassayed their extract on their own animals it is impossible to make any comparison on this basis. It is of course possible that the extract caused rapid luteinization in their animals with a subsequent prolongation of gestation due to the induced functional corpora lutea. In the experiments reported here, the rôle of antuitrin-S seemed to be that of an abortient rather than to prolong gestation.

In regard to the ovaries, the presence of abnormally large cystic and atretic follicles indicated that in these cases the induced corpora lutea had not inhibited follicular growth. If, as Engle (17) states, pregnancy urine contains only LH, then it must be assumed that the development of large follicles following antuitrin-S injections was due to the FSH released from the animal's own pituitary.

It is shown in this study that the mere presence of corpora lutea does not mean that they are necessarily functional. The evidence from these experiments indicates that parturition is not entirely due to a lack of secretion of the corpora of pregnancy, and that the utero-placental complex plays an important rôle in maintaining and terminating pregnancy.

SUMMARY

The effects of antuitrin-S, progestin, and a vital dye (dianil blue 2R) on late pregnancy were investigated in 170 animals. The following conclusions have been drawn.

Antuitrin-S, when injected in dosages of 50 to 75 R.U. on the 18th day of pregnancy, resulted in birth, prematurely or at term, of living, dead, or living and dead

fetuses in the case of 93.9% of these animals. The fetuses which were born alive were usually not viable. In none of these cases was parturition postponed or prolonged.

Dosages of 150 to 200 R.U. made earlier than the 19th day usually resulted in parturition being postponed and in some instances prolonged from 1 to 5 days. The fetuses died in utero before or at term, and in some cases even death of the mother resulted. The induced corpora are not regarded as being responsible for postponed and prolonged parturition.

Injections of 50 to 200 R.U. made on the 19th, 20th and 21st days usually resulted in the birth of viable fetuses at term.

Progestin in doses up to 5 I.U. given in late pregnancy did not appreciably affect the length of gestation or survival of the fetuses.

The vital dye, dianil blue 2R, when injected from the 14th to the 17th days, in doses of 4 to 5 cc. daily, resulted in death of the fetuses in the majority of cases, and in some cases death of the mother. These results are considered to be due to the toxicity of the product.

The physiological phenomena of premature birth, postponed and prolonged parturition were correlated with the condition of the ovaries, placentae, and uteri as revealed by histological studies. The results of these studies lead the writer to believe that the utero-placental complex plays an important rôle in maintaining pregnancy and in determining the time of parturition, and that birth is not entirely due to a lack of secretion by the corpora lutea of pregnancy.

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CREATINE-CREATININE METABOLISM AND THE HORMONES

III. EFFECT OF PARENTERAL INJECTION OF CREATINE AND CREATININE WITH THE SEX HORMONES UPON CREATINE-CREATININE EXCRETION IN NORMAL ANIMALS

HOWARD H. BEARD AND ERNEST J. JACOB

From the Department of Biochemistry, Louisiana State University School of Medicine

NEW ORLEANS, LOUISIANA

A RECENT TEXT on biochemistry states that creatinine is a useless waste product and when ingested most of it is excreted into the urine. It further states that about one-third of the body creatine is changed into creatinine and that the sex hormones are involved in this process (1). These views have long been accepted in the field of creatine-creatinine metabolism and are derived chiefly from the fact that women and children are known to excrete some creatine while men usually do not. The sex hormones, however, do not effect the change of creatine into creatinine, even if creatine were transformed into creatinine. This is shown by the fact that almost as much creatinine is excreted in women as compared to men in 24-hour periods. We have shown that the so-called constancy in creatinine elimination in both sexes is not due to body weight or muscle weight but to a uniform protein and water intake resulting in a uniform creatine-creatinine metabolism and creatinine excretion (2, 3). It is possible that theelin and progestin cause more creatinuria in the female than does testosterone in the male.

The above views are also contrary to much published evidence from this laboratory that creatine is not changed into creatinine in the body of the man and rat but, in the latter, causes its retention instead. Furthermore it was definitely shown that creatinine injections in the rat and ingestion in man greatly stimulated the excretion of creatine, and in some cases of creatinine, in the urine (4). We have also been unable to detect a sex difference in creatine-creatinine excretion in the rat. This view is logical since there is very little difference in the chemical structure between the male and female sex hormones in spite of the great differences in their physiological functions.

In view of the above conflicting ideas of the effect of the sex hormones on creatine-creatinine metabolism it was decided to make a further study of this question. The results obtained are given in figures 1 to 3 and in table 1.

EXPERIMENTAL

Different amounts of creatine and creatinine, with and without the sex hormones, were injected into normal rats and the effect of this procedure on creatine-creatinine excretion was observed. The technic has been described in the first paper of this series (5). When 0.2 mg. of theelol in oil is added to 12 cc. of alkaline picrate an equivalent of 0.18 mg. creatinine was observed. It is unlikely, however, that this small dose of theelol would have been excreted so this small error is of no significance upon the results obtained. Rats 1 to 9 (fig. 1) were males and rats 1 to 10 (fig. 2) were females.

TABLE 1. THE EFFECT OF CREATININE, WITH AND WITHOUT THE SEX HORMONES, UPON CREATINE EXCRETION IN NORMAL RATS

Creatinine	Sex hormones	Creatinine	Sex hormones	Experiment	No. of rats	Total amount of creatine, as creatinine, excreted
mg.	mg.	mg.	mg. ♂ mg. ♀	days		mg.
25				16	2	29.3
	10			16	2	84.0
		25	10	24	2	152.0
50				16	2	40.1
	10			16	2	87.0
		50	10	24	2	125.0
75				16	2	30.0
	10			16	2	67.0
		75	10	24	2	101.0
100				16	2	40.0
	0.2			16	2	71.0
		100	0.2	24	2	130.0
150				16	2	41.0
	0.2			16	2	64.0
		100	0.2	24	2	115.0
200				16	2	60.0
	0.2			16	2	34.0
		100	0.2	24	2	80.0

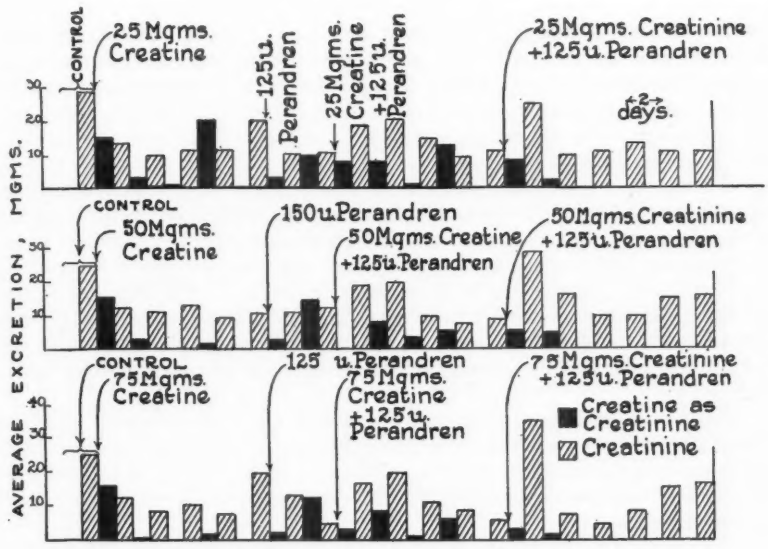

FIG. 1. EFFECT OF MALE SEX HORMONE ON CREATINE-CREATININE EXCRETION.

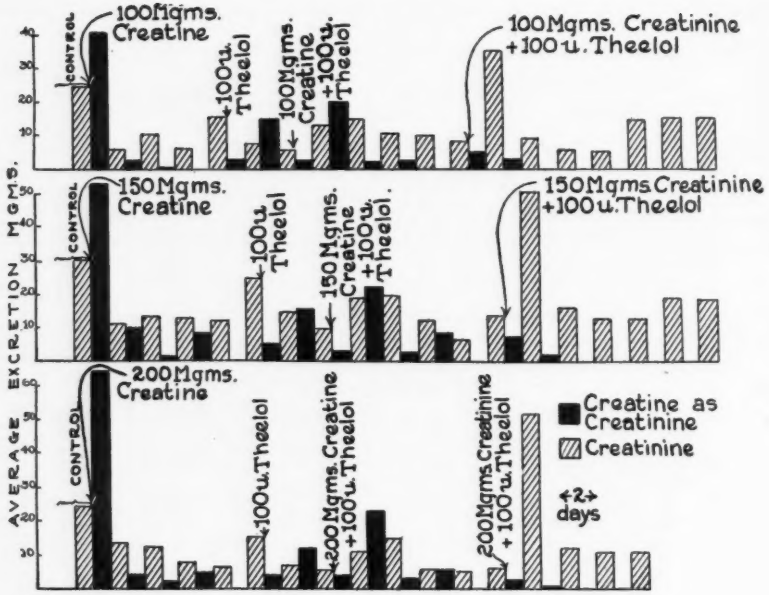

Fig. 2. Effect of female sex hormone on creatine-creatinine excretion.

RESULTS AND DISCUSSION

The injection of creatine in doses from 25 to 200 mg. does not increase the creatinine output in the rat but again causes its retention instead. The injection of the male and female sex hormones alone causes increases in creatine but not in creatinine excretion. When the sex hormones and creatine are injected together increases in creatine, but not in creatinine excretion, occurred. All of these results confirm those we have recently published (5).

When doses of creatinine from 25 to 200 mg. were injected after mixing with the oily solutions of the sex hormones, only slight increases in creatine excretion occurred. An emulsion was formed in these cases which probably nullified the effect of the sex hormones in this connection. The injected creatinine was promptly excreted.

These results are contrary to those published by Jacob and Beard (4) in that creatinine did not stimulate the excretion of creatine. The experiment was, therefore, repeated. In this case the sex hormones were injected into one flank of the animal and creatinine into the other about 1 hour later. The results obtained are given in figure 3.

Under these conditions the separate injection of creatinine and the sex hormones caused a retention of creatinine for 9 days following injection, after which most of it was excreted. In confirmation of our previous results it is clear that the retention of creatinine results in a stimulation of creatine excretion, which was again obtained in this experiment.

The results of further experiments listed in table 1 give a comparison of the effect of the sex hormones, with and without creatinine, on creatine excretion. In practically all cases there was again a retention of creatinine for 9 to 12 days and a summa-

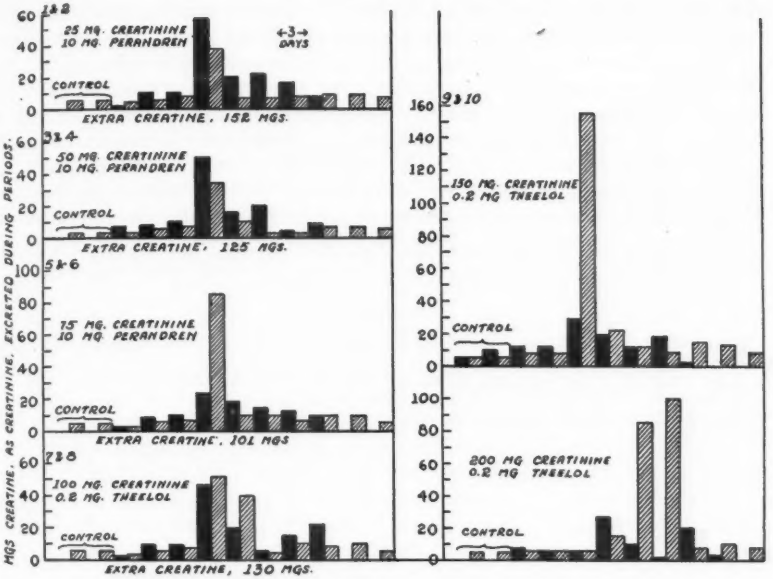

Fig. 3. Effect of parenteral injection of creatinine, with and without the sex hormones, upon creatine excretion.

tion of creatine excretion when the sex hormones are injected with creatinine, as compared to the creatine excretion obtained when the sex hormones and creatinine are injected separately and at different times in the same animals. These results show clearly that the stimulating effect of creatinine on creatine excretion is increased when the sex hormones are injected at the same time.

The difference in experimental results in regard to creatine excretion listed above shows the great importance of experimental technic used in studies of this nature. The sex hormones should always be injected separately from creatinine if increases in creatine excretion are to be observed. The chief point of interest in the results of the present study is that the sex hormones assist creatinine in its 'stimulating effect' on creatine excretion.

SUMMARY

Different amounts of creatine or creatinine, with and without the male (testosterone propionate) and female hormone (theelol) were injected into normal rats and the effect of this treatment upon the creatine-creatinine excretion noted. The results obtained were as follows.

Injection of creatine in doses from 25 to 200 mg. does not increase the excretion of creatinine, but causes its retention instead.

The injection of either the male or female sex hormone alone causes increases in creatine, but not in creatinine, excretion.

The injection of creatine and the sex hormones does not lead to any increase in creatinine excretion.

The injection of creatinine in doses from 25 to 200 mg. mixed with the oily solutions of the sex hormones causes no increase in creatine excretion. If, however, creatinine and the sex hormones are injected separately, increases in creatine excre-

tion were obtained. There was a summation of creatine excretion obtained when creatinine and the sex hormones were injected at different times in the same animal.

The sex hormones caused creatinine retention for 9 days after injection. It may, therefore, be concluded that (*a*), creatine is not changed into creatinine in the rat; (*b*), the sex hormones do not assist in the creatine-creatinine transformation or in creatinine excretion; (*c*), under proper experimental conditions the injection of creatinine stimulates the excretion of creatine, and this function of creatinine is increased after the injection of the male or female sex hormone; (*d*), no difference in the effect of the sex hormones on creatine-creatinine metabolism in rats has been observed.

We wish to express our thanks to the Ciba Pharmaceutical Products, Inc., Summit, N. J., for the samples of perandren and to Parke, Davis & Co., for the samples of theelol, used in these studies.

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CREATINE-CREATININE METABOLISM AND THE HORMONES

IV. EFFECT OF PARENTERAL INJECTION OF THE SEX HORMONES, WITH AND WITHOUT SALINE AND CREATININE, UPON CREATINE-CREATININE EXCRETION IN THE 90-DAY CASTRATE RAT

HOWARD H. BEARD AND ERNEST J. JACOB

From the Department of Biochemistry, Louisiana State University School of Medicine

NEW ORLEANS, LOUISIANA

IN THE FIRST PAPER of this series (1) it was shown that parenteral injection of theelin or testosterone propionate caused a more intense creatinuria in the castrate than in the same animal before castration. This would indicate that these hormones either assisted in the formation or excretion of creatine. It is also possible that they were assisting in the transformation of creatinine into creatine through their water and salt retaining effects (2) and to the fact that, in some instances, creatinine retention occurred.

These results were obtained in the first few weeks following removal of the ovaries and testes. We desired to obtain further information upon creatine-creatinine excretion in the 90-day castrate rat. It is possible that in this preparation a somewhat different creatinine-creatine equilibrium would be established, the results of which may differ from those obtained above with normal animals and recent castrates. The results obtained are given in this paper.

EXPERIMENTAL

The technic used was described in the first paper of the series (1). The animals

TABLE 1. THE EFFECT OF PARENTERAL INJECTION OF THE SEX HORMONES, WITH AND WITHOUT SALINE AND CREATININE, UPON CREATINE-CREATININE EXCRETION IN 90-DAY CASTRATE RATS

Rat No.	Saline	Creatinine	Perandren	Theelol	Experiment	Total excretion	
						Creatinine	Creatine (as creatinine)
	cc.	mg.	U	U	days	mg.	mg.
1	2		250		16	73	49
2	2	20	250		16	109	49
3	4		250		16	114	69
4	4	40	250		16	97	41
5	6		500		16	97	47
6	6	60	500		16	118	42
7	8		500		16	128	55
8	8	80		100	20	207	62
9	10			100	20	143	61
10	10	100		200	18	194	43
11	12			200	18	168	56
12	12	120		400	18	208	61
13	12	120		400	18	202	64

were castrated under ether anesthesia. Animals 1 to 7 were males and 8 to 13 females. Ninety days following castration different amounts of physiological saline with the male or female sex hormone were injected into one animal; creatinine in addition was injected into the next, and so on. The results obtained from this procedure upon creatine-creatinine excretion are listed in table 1.

RESULTS AND DISCUSSION

The results obtained show that, in these older castrates, the injection of the male or female sex hormones did not cause increases in creatine excretion, above that obtained from the injection of saline alone. This finding confirms our results published for the 180-day castrate rat (3). The injection of creatinine likewise had no effect upon creatine excretion except in one case. The creatinuria here, as obtained above with the hormones, was due to the injection of saline alone (3).

It has been observed in this laboratory that the injection of creatinine from 25 to 200 mg. in normal rats caused an increase in creatine excretion ranging from 23 to 60 mg., an average of 37 mg. In a more recent study increases in creatine excretion ranged from 100 to 150 mg. when creatinine and the sex hormones are injected into the normal animal at the same time (4). This shows that these hormones increased the 'stimulating effect' of creatinine on creatine excretion (5).

Since the effect of the sex hormones and creatinine upon creatine excretion are abolished in the 90 and 180-day castrate rat as compared to their effect in recent castrates and normal animals, it is evident that, in these older castrates, changes in some of the other endocrine glands must have occurred which blocked the usual effects of the sex hormones and creatinine upon creatine-creatinine metabolism. It is of much interest that injection of saline alone under these conditions results in creatinuria. The evidence does not point to any change in kidney function.

A brief summary of our results of the effect of the sex hormones upon creatine-creatinine metabolism follows.

(*a*) Normal rat+sex hormones (testosterone propionate, theelin, progestin) or antuitrin-T and G)→Creatinuria (not due to creatinine but possibly to water and salt retention) (1).

(*b*) Normal rat+creatinine→Creatinuria (due to the hydrolysis of creatinine to creatine, and also to the stimulating effect of creatinine upon creatine excretion) (5).

(*c*) Normal rat+sex hormones+creatinine→Creatinuria (the stimulating effect of creatinine upon creatine excretion is increased in the presence of either the male or female sex hormone) (4).

(*d*) Recent castrates→Temporary creatinuria (some of which is due to the anesthetic and trauma of the operation) (6).

(*e*) Recent castrates+sex hormones (theelin, testosterone propionate and progestin, as well as antuitrin-T and G)→Creatinuria (which is more intense in the castrated as compared to the same animal before castration) (1, 7).

(*f*) Three and 6 months castrates+sex hormones+creatinine→Creatinuria (due to effect of injected saline alone).

(*g*) Normal, recent and old castrate animals+saline→Creatinuria (due to the hydrolysis of creatinine to creatine) (3).

SUMMARY

The sex hormones, testosterone propionate and theelol, with and without saline and creatinine, were injected into 90-day castrate male and female rats and the effect of this procedure upon creatine-creatinine excretion in the urine was studied. No

effect was observed under these conditions upon the excretion of creatine or creatinine which is in contrast to the creatine excreted in the normal animal or recent castrate under similar experimental conditions. The creatinuria observed was due to the effect of saline alone. It was suggested that secondary changes in some of the other endocrine glands may account for these observations. All results in this field are briefly summarized.

We wish to express our appreciation to the Ciba Pharmaceutical Products, Inc., Summit, N. J., for the samples of perandren and to Parke, Davis & Co., for the samples of theelol, used in these studies.

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THE EFFECT OF MULTIPLE INJECTIONS OF PROGESTERONE ON THE TURGESCENT PERINEUM OF THE BABOON (PAPIO PORCARIUS)

JOSEPH GILLMAN

From the Department of Anatomy, University of the Witwatersrand
JOHANNESBURG, SOUTH AFRICA

THE PERINEUM of the adult female chacma baboon has been shown to undergo rhythmical alterations in size during the menstrual cycle (1, 2, 3). These fluctuations have been measured and recorded daily and correlated with the vaginal smear (4). Parkes and Zuckerman (5) were the first to show that the turgescence of the perineum in baboons is due to the presence of estrone. This has been confirmed by Gillman in both adult and prepubertal baboons (6, 7).

The factors responsible for perineal deturgescence and menstruation are less definitely known. Allen (8, 9) and Corner (10) have suggested that the withdrawal of estrone is responsible for menstruation. This has been largely supported by Zuckerman (11). Fluhman (12) maintained on valid evidence that the 'estrin privea' hypothesis of Allen and Corner was inadequate to explain the onset of bleeding. Parkes and Zuckerman have shown that in a spayed baboon estrone would cause the perineum to turgesce, and that deturgescence occurred on the withdrawal of estrone. From this it might be deduced that perineal deturgescence is a negative phenomenon due simply to a drop in the estrone concentration in the blood. It has been shown in a previous study (7) that the perineal deturgescence that supervenes in an immature baboon after a course of estrone is obviously due to 'estrone privea' but that large injections of estrone early in the second half of the cycle in an adult baboon failed to cause a deturgescent perineum to turgesce.

For this and other reasons it was suggested that in immature baboons deturgescence might be due to estrone withdrawal; but that in normal adult female baboons whose cycle was regular, perineal deturgescence was not dependent entirely on a lowered estrone threshood.

If perineal deturgescence in adult baboons is a positive and not a negative phenomenon then there must be some other hormone whose presence in the blood in sufficient concentration actively produces perineal deturgescence once the perineum is in the stage of turgescence. One such substance has been found in progesterone.

The present paper is a report of the results obtained by multiple injections of known quantities of progesterone early in the stage of perineal turgescence.

MATERIAL AND METHODS

The baboons used in this investigation were mature animals, which possessed permanent teeth including third molars. These animals had been under constant observation for periods varying from 6 months to 4 years. The menstrual rhythm of each animal had been observed throughout and was regular. The progesterone used was kindly supplied by Messrs. Schering Co. under the trade name of proluton. Injections were made intramuscularly into the thigh, care being taken that alternate limbs were used on each day. The details of the experiments are summarized in table 1.

TABLE I

Experiment No.	Length of normal cycle	Injection of progesterone (proluton)	Total amt. progesterone given	Total period over which progesterone was given	Time of bleeding after last injection of progesterone	Length of experimental cycle
	days		mg.	days	hr.	days
1	38	Day of cycle 4 5 6 7 8 9 10 11 12 13 14 Dose in mg. 3 3 5 5 5 5 5 5 5 5 5	51	11	68	18
2	35	Day of cycle 8 9 10 11 12 14 16 17 18 19 Dose in mg. 5 4 2.5 1.5 2.5 4 3 2.5 2.5 2.5	30	12	96	23
3	38	Day of cycle 8 9 10 11 12 13 Dose in mg. 5 5 5 5 5 5	30	6	90	17
4	38	Day of cycle 8 10 14 Dose in mg. 5 5 5	15	7	144	20
5	38	Day of cycle 5 9 Dose in mg. 5 5	10	5	nil	47
5a	38	Day of cycle 5 9 Dose in mg. 5 5	10	5	nil	45
6	42	Day of cycle 9 12 Dose in mg. 7½ 7½	15	4	120	17

RESULTS

First experiment. The first series of experiments was planned to determine what the effect of daily injections of progesterone would be on the turgescent perineum of the baboon. The administration of 51 mg. of progesterone over 11 days commencing on the 4th day of the cycle; i.e. one day after the disappearance of external bleeding (table 1), produced a characteristic effect on the perineal rhythm. On the 7th day of the cycle or 3 days after the initial injection the perineum commenced to deturgesce and reached the resting state on the 14th day. The perineum remained in a state of rest until the onset of bleeding. This occurred on the 68th hour after the last injection of progesterone.

Second experiment. This experiment aimed at discovering whether half the quantity of progesterone used in the first experiment given over the same period would produce a similar result. A little more than half the quantity (30 mg.) of progesterone was given over a period of 12 days starting on the 7th day of the cycle. The perineum rapidly underwent deturgescence and reached the resting level on the 12th day of the cycle. This resting state was maintained throughout the entire period of progesterone treatment. The last injection was given on the 19th day of the cycle and bleeding supervened on the 23rd day, i.e. 96 hours after the final administration of progesterone. The bleeding lasted for 4 days. Thus 30 mg. progesterone was able to produce perineal deturgescence in 6 days and precipitate bleeding 96 hours after the last injection.

Third experiment. This experiment was performed to determine the effect of the same dose of progesterone but given for half the length of time (i.e. 6 days instead of 12 days) as in the second experiment. The injections were started on the 8th day, perineal deturgescence was complete on the 14th day. Perineal rest was maintained until the onset of bleeding which supervened on the 17th day of the cycle; i.e. 90 hours after the final injection. Thus by giving 30 mg. of progesterone over 6 days it has been possible to produce perineal deturgescence within 6 days and also bleeding on the 90th hour after withdrawal. In this latter case it should also be noted that the cycle was shortened by no less than 21 days.

Fourth experiment. The object of this experiment was to determine whether half the reduced dose of progesterone used in the third experiment but given over the same period would produce the same effect on the perineum. Three injections of 5 mg. progesterone were given over 7 days commencing on the 8th day of the cycle. Deturgescence once more was complete within 6 days i.e. in this case on the 14th day. Bleeding occurred 144 hours after the last injection i.e. on the 20th day. Thus on giving 15 mg. of progesterone over 7 days perineal deturgescence supervened within 6 days and bleeding 144 hours after the last injection. The cycle was shortened by 18 days.

Fifth experiment. The object of this experiment was to determine whether a still lesser total quantity (10 mg.) of progesterone given over only 5 days would be adequate to cause perineal deturgescence and precipitate bleeding. By giving 2 injections of 5 mg. progesterone at a 4-day interval starting on the 5th day of the cycle perineal deturgescence was complete within 6 days. But in this animal no bleeding occurred externally or vaginally, as verified by vaginal lavage. The perineum, however, remained in a state of rest for 2 days and on the 13th day, i.e. on the 5th day after the last injection the perineum began to turgesce and so to exhibit its normal upward menstrual rhythm. Bleeding first occurred only after this succeeding and virtually normal cycle had occurred, i.e. on the 47th day after the onset of the original cycle which had been experimentally interfered with. In other words in this animal the cycle was postponed for 9 days. Thus 10 mg. of progesterone given in 2 doses at an interval of 4 days, the first injection being given on the 5th day of the cycle caused a deturgescence within 6 days after the initial dose of progesterone but was unable to produce bleeding. The perineum then underwent its rhythm of turgescence, deturgescence and rest in an approximately normal fashion.

Sixth experiment. The fifth experiment revealed that 10 mg. of progesterone was unable to produce bleeding whereas in the 4th experiment 15 mg. given in 3 doses was able to produce bleeding. The object of this experiment was to determine whether a total of 15 mg. given in 2 doses of 7½ mg. each would be able to produce bleeding. The injections were started on the 9th day of the cycle. The second and last injection was given on the 12th day. The perineum came down in the usual manner and bleeding occurred on the 17th day of the cycle, i.e. 15 mg. of progesterone given in 2 doses are sufficient to produce bleeding.

DISCUSSION

A. *Antagonism of estrone and progesterone.* The antagonistic action of estrone and progesterone has been known for a decade (11, 13–20). With the exception of Hisaw's and Zuckerman's contribution concerning *Macacus rhesus*, all the above investigations have been performed on rabbits, rats or mice.

In *Papio porcarius* progesterone obviously antagonizes the action of estrone as gauged by its deturgescent effect on the perineum whose turgescence is evoked by estrone. The external perineal indicator has made it possible here to ascertain facts which hitherto in other animals could only be appreciated after destructive histological examination. Even then histological sections can only give a qualitative clue to the state of the genital organs at a specific point in time. By means of perineal measurement, however, quantitative reactions to the hormones can be followed hourly, daily, monthly and even annually without subjecting the animal to violent operative procedures which are known to complicate the issue (6).

We therefore know that progesterone in the turgescent phase of the perineal cycle produces an effect within 48 hours at the latest and usually within 24 hours provided the dose is adequate. The perineum returns rapidly to its resting state

within 4 to 6 days. Thus it takes 4 to 6 days after administration of progesterone before the progesterone ceases to exert its effect on the perineum.

This proves conclusively that the deturgescence of the perineum which characterizes the second half of the cycle is a positive phenomenon due in normally ovulating baboons to the presence of progesterone and not only a negative phenomenon resulting from the absence of estrone. Progesterone may cause the effect by inactivating the estrone, but there can be no doubt that by giving progesterone in the first part of the cycle it is responsible for producing deturgescence directly by its own effect on the perineum or indirectly by eliminating or inactivating estrone produced at this time by the baboon's ovaries.

B. Delayed effects of progesterone. Apart from affording this critical information the perineal indicator has also revealed the delayed effects of the progesterone. An examination of table 1 shows that it takes at least 96 to 120 hours after the last dose of progesterone before the ovaries of the baboon can regenerate sufficient estrone to exert its turgescent effect once more on the perineum irrespective of the dose of progesterone used and irrespective of whether bleeding does supervene or not. The practical clinical importance of this observation lies in the fact that the effect of progesterone wears off within 4 or 5 days. It is therefore unnecessary to give daily injections of progesterone if the dose is adequate in the first instance. Also in experimental procedures progesterone can be safely given at 3-day intervals in the baboon and not unnecessarily every day.

The effect of progesterone on the succeeding cycles has been followed by using the perineum as an indicator. In all the animals where the dosage has been adequate to result in bleeding the next cycle has not been materially affected although the total length of the cycle may be shortened by 4 to 6 days. The second cycle following the experimental one can therefore be regarded as normal.

Where bleeding has not been induced the cycle has been prolonged by 9 days. This lengthening of the cycle is roughly equivalent in length to the period of depression of the turgescent perineum caused by the injections of progesterone.

Once deturgescence of the perineum has been precipitated in the animals so far used in these experiments the deturgescence continues for 4 to 8 days until the perineum reaches the level which can be regarded as the resting state for the animal concerned.

C. The induction of bleeding after progesterone withdrawal. The remarkable fact which emerges from these experiments is the early precipitation of bleeding at a stage in the cycle when the uterus has scarcely recovered from its recent menstruation. This effect of progesterone, known to me for over two years, was recently shown by Zondek and Rozin (21) to exist in the human female, after 50 mg. of progesterone was administered over a period of 5 days i.e. 10 mg. daily. In these experiments on baboons it was possible to produce bleeding by injections of only 15 mg. given in 3 injections at 2-day intervals. Total doses smaller than 10 mg. were insufficient to produce bleeding.

In a previous publication (22) we have shown that less progesterone is required by the baboon than by the monkey, to inactivate estrone, and that the monkey requires less than the rabbit and the rabbit less than the mouse. We suggested at the time that it is very likely that the human female is much more sensitive to progesterone than the baboon. It seems on this basis that the doses used by Zondek and Rozin in the human female were far in excess of the actual requirements to precipitate bleeding. Experiments are now proceeding in our laboratories to verify this hypothesis.

D. Relation of the dose of progesterone to the onset of withdrawal bleeding. The relation between the dosage of progesterone and the time of onset of bleeding needs closer

examination. From table 1 it is clear that after withdrawal the bleeding occurs much earlier when 30 mg. have been used than with 15 mg. Again the onset of bleeding after giving 15 mg. of progesterone is delayed 2½ times longer when 51 mg. have been given. It is not the spacing of the injections which delays the bleeding but rather the total dose of progesterone utilized. This is clearly shown in experiments 3 and 4, where the same dosage, namely 30 mg., was used, but in one case spread over 12 days and in the other over 6 days and still the bleeding occurred at the same time, i.e. 90–96 hours after the last injection.

Thus a very close relation between the dose of progesterone and the time of onset of bleeding after the last injection obviously exists.

E. Progesterone privea and estrone privea as a cause of bleeding. The withdrawal of estrone has been put forward as the cause for uterine bleeding but from the above experiments there is no doubt that the bleeding will also occur if progesterone is withdrawn. The continued administration of progesterone prevents the onset of bleeding (experiment 1). It is not possible as yet to explain the mechanism responsible for initiating premature bleeding after the withdrawal of progesterone which has been given in large doses early in the cycle.

Smith, Smith and Pincus (23) in their concluding remarks on their experiments on the human female and on rabbits state that "progesterone prevents the destruction of estrin and facilitates the conversion of estrone into physiologically less active estriol" and offer evidence in support of the assumption that progesterone deficiency pertains prior to menstruation.

Thus it seems that the onset of bleeding in normal animals is due to the absence of progesterone rather than to the absence of estrone. Frank and Goldberger (24) have shown that there is a rise in estrone in the blood before menstruation, and Gillman (7) has shown that the injection of large quantities of estrone cannot cause turgescence in a perineum that has become deturgescent and that the normal progress of the cycle cannot be impeded by the presence of large quantities of estrone when given in the second part of the deturgescent phase of the cycle.

There is sufficient evidence available to show beyond a reasonable doubt that the withdrawal of estrone from castrated animals will cause bleeding, but there is equally sufficient evidence to show that a uterus can be made to bleed by administering progesterone when the uterus has been very adequately or even inadequately prepared by estrone. Thus there are two factors responsible for bleeding, namely the withdrawal of estrone and the withdrawal of progesterone. The progesterone alone cannot produce bleeding, and it is very likely that progesterone inactivates the estrone and causes bleeding by estrone withdrawal. The bleeding is much quicker after progesterone withdrawal than after estrone withdrawal alone. This is shown by experiments on prepubertal baboons (unpublished data). When estrone is given to prepubertal baboons the bleeding occurs only 10 to 16 days after withdrawal (25), whereas as we have seen from the above experiments, the giving of progesterone to adult animals who have produced their own estrone for only a few days, as gauged by the perineal reaction, the bleeding occurs 60 hours after withdrawal. This means that the withdrawal of progesterone causes an alteration in the uterine vessels resulting in bleeding in a shorter time, (60 hours) after progesterone as compared with 10 to 16 days with estrone alone.

The exact correlation between progesterone dosage and estrone in relation to bleeding will be incorporated in a later communication.

F. Production of short cycles by progesterone. Since bleeding follows soon after the withdrawal of progesterone it has been possible to produce short cycles artificially. In one case (experiment 3) the cycle was only 17 days instead of 35 whereas in another case (experiment 4) the cycle was only 20 days instead of 38 days.

On the other hand by giving doses less than 15 mg. which are insufficient to cause bleeding, but adequate to inhibit the turgescent phase temporarily, it has been possible to prolong the cycle by 9 days. Thus by giving small doses of progesterone at properly spaced intervals it is possible to prolong the cycle indefinitely.

SUMMARY

The perineum of the baboon has been shown to be a sensitive indicator in experiments involving the use of the female sex hormones. Deturgescence in the normal adult female baboon is a positive phenomenon due to the presence of progesterone and not only to the absence of estrone. Progesterone administered in total doses of 10 mg. or less will cause a depression of the turgescent perineum. The artificially induced perineal deturgescence lasts for 4 to 6 days and is not succeeded by bleeding. Progesterone administered in total doses exceeding 15 mg. produces perineal deturgescence and is succeeded by bleeding after withdrawal of progesterone. By repeated injections of progesterone in the turgescent or first part of the cycle it is possible to prolong the cycle indefinitely or by early withdrawal it is possible to shorten the cycle to just half the normal length for the animal concerned. Adequate quantities of progesterone given in the first part of the cycle can precipitate bleeding at fixed intervals within limits after the withdrawal of progesterone.

The time of onset of bleeding after withdrawal is dependent entirely upon the total quantity of progesterone used. Large total quantities (50 mg.) produce bleeding 60 hours after withdrawal, small total quantities (15 mg.) will precipitate bleeding 144 hours after the last injection. Bleeding follows 4 to 6 times more rapidly after progesterone withdrawal than after estrone withdrawal alone. The action of progesterone depends on the preliminary or simultaneous treatment with estrone. Progesterone (proluton) exerts its effect 6 to 48 hours after injection and its action wears off in 4 to 6 days.

I wish to acknowledge my indebtedness to Professor Dart for his help and criticism. My thanks are also due to Dr. H. B. Stein who has materially assisted me in planning these and other experiments. Messrs. Schering & Co. have very generously supplied the progesterone (proluton).

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THE EFFECT OF TESTOSTERONE, ESTRONE AND ESTRADIOL APPLIED LOCALLY TO THE PENIS OF THE RAT[1]

H. S. WIGODSKY AND R. R. GREENE

From the Department of Physiology and Pharmacology, Northwestern University Medical School

CHICAGO, ILLINOIS

THE AUTHORS HAVE previously reported that the application of a testosterone ointment (75γ daily for 22 days) to the penes of castrate rats causes growth of the penis (1). The growth was of the same order as that found in untreated normal litter mates and in castrated litter mates with daily subcutaneous injections of the same amount of testosterone in peanut oil. The purpose of the present experiment was to determine the effect of smaller doses of testosterone and shorter periods of treatment. Alcohol has also been used as the solvent and the effects of estrone and estradiol have been observed.

The procedure used was essentially that previously described. The only variation has been that in some groups the period of treatment has been shortened to 10 days. As in the previous experiment, all comparisons were made between litter mates. The controls in each group were treated daily with the solvent only of that particular group. All animals were castrated and treatment started at the age of 24 days. The results are shown in table 1.

RESULTS

Group I. The treated animals received 7.5γ of testosterone in alcohol to the penis daily for 22 days. The penes of the treated animals averaged 6% longer and weighed 41% more than those of the controls. The prostatic-seminal vesicle complex averaged 14% heavier than those of the controls.

Group II. In this group the treated animals received 7.5γ of the testosterone in alcohol daily to the penis for 10 days. The increase in penis length, penis weight, and prostatic-seminal vesicle weight over that of the controls averaged 10%, 50%, and 32% respectively. This is a slightly greater increase in penis length and weight, and more than twice as great an increase in prostatic-seminal vesicle weight than resulted from the same dose of testosterone applied also in alcohol solution for 22 days (Group I).

Group III. The treated animals received local applications to the penis of 7.5γ of testosterone in a lanolin ointment daily for 22 days. This was 1/10th the dose used in the previous report. The penes of the treated animals averaged 6% longer and weighed 23% more than the lanolin treated controls. The prostatic-seminal vesicle complex averaged 17% heavier than the controls. This is about 50% of the increase noted in Group II when the same dose of testosterone was dissolved in alcohol and applied for only 10 days. The penis length and prostatic-seminal vesicle complex

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The testosterone and estradiol (ovocylin) were furnished through the courtesy of Ciba Pharmaceutical Products, Inc. The estrone (estrolin) was furnished through the courtesy of the Lakeside Laboratories.

weight are approximately the same as Group I. However, the increase in penis weight in this group is only 50% of that noted in Group I where the same dose of testosterone was administered for the same length of time, but with alcohol as a solvent.

Group IV. In order to determine the efficacy of local application of testosterone as compared to subcutaneous injections, 7.5γ of testosterone dissolved in peanut oil was injected subcutaneously daily for 10 days in this group. The average increase in penis length and penis weight over the controls was 6% and 42% respectively. The increase in weight of the prostatic-seminal vesicle complex was 85%. The increase

TABLE I

Group	Treatment	No. of animals	Days of treatment	Ave. wt. gm.	Ave. length of penis, mm.	Ave. wt. of penis, mg.	Ave. wt. prostate-seminal vesicles, mm.	Ave. % wt. increase length of penis	Ave. % increase weight of penis	Ave. % increase wt. of pros.-sem. vesicles
I	controls-alcohol	6	22	79	17±0.27	37±0.58	56±2.5			
	7.5γ testosterone daily in alcohol, local applic.	7	22	60	18±0.41	52±1.4	64±2.8	6	41	14
II	controls-alcohol	8	10	69	16±0.31	38±1.3	47±1.1			
	7.5γ testosterone daily in alcohol, local applic.	9	10	66	18±0.12	57±2.2	62±2.7	10	50	32
III	controls-lanolin	8	22	97	17±0.17	44±0.52	47±0.88			
	7.5γ testosterone daily in lanolin, local applic.	9	22	101	18±0.24	54±1.30	55±1.60	6	23	17
IV	controls-peanut oil	9	10	58	16±0.32	36±1.0	40±1.2			
	7.5γ testosterone daily in pnt. oil, subcut. inject.	9	10	59	17±0.08	51±1.9	74±2.3	6	42	85
V	controls-alcohol	9	22	110	18±0.24	43±1.5	53±1.6			
	1γ estradiol daily in alcohol, local application	9	22	107	19±0.17	47±1.3	122±2.6	5	9	130
VI	controls-alcohol	10	10	60	16±0.2	36±1.0	42±3.0			
	5γ estradiol daily in alcohol, local application	11	10	65	15±0.2	37±1.0	77±2.0	−6	3	83
VII	controls-alcohol	20	10	63	16±0.12	38±0.90	43±1.71			
	5γ estrone daily in alcohol, local application	21	10	63	16±0.02	39±0.80	58±1.93	0	3	35

$$\text{P.E.} = 0.6745 \pm \sqrt{\frac{\Sigma\delta^2}{n(n-2)}}$$

in penis length and weight is slightly below that resulting from local application of the hormone in an alcoholic solution for the same period of time (Group II), but the increase in the weight of the prostatic-seminal vesicle complex is almost three times that observed in this same group.

Group V. In order to determine the effect of estrogenic substances on the penis when applied locally, 1γ of estradiol, dissolved in alcohol, was applied daily to the penes of this group for 22 days. This resulted in an increase of 5% in the length and 9% in the weight of the penes of the treated animals as compared to the controls. However the difference in the lengths and weights of the penes of the control and treated animals is not statistically significant. The prostatic-seminal vesicle complex averaged 130% heavier than those of the control animals.

Group VI. A larger dose of estradiol, 5γ, applied for only 10 days resulted in a decreased penis length in the treated animals (6%). The penis weight was 3% greater in the treated animals, but this was not statistically significant. The increase in prostatic-seminal vesicle weight was 83%. This is less than ½ that observed in Group V where 1γ of estradiol was applied for 22 days.

Group VII. Another estrogenic substance, estrone, was applied locally for 10 days in 5γ doses dissolved in alcohol. The resulting average increase in penis length and weight, and weight of the prostatic-seminal vesicle complex was 0%, 3%, and 35% respectively.

DISCUSSION

Previous workers have noted enlargement of the prostate and seminal vesicles of castrated animals when treated with estrogens. This weight increase is due to stimulation of the connective tissue stroma of these structures. It is known that percutaneously applied estrogens are absorbed and have systemic effects (2–6). In the estrogen treated animals in our series, as was expected, the prostatic-seminal vesicle complex was significantly heavier than in the control animals. Grossly, the increase in weight seemed to be due principally to an increase in size of the seminal vesicles. The estrogens, however, had no significant effect on the length or weight of the penis.

Percutaneously applied estrogens and androgens are known to be more effectively absorbed in the rat when they are in alcoholic rather than in oil or ointment solutions (3, 5, 7). A similar effect was obtained in this procedure. A greater penis weight increase was obtained from testosterone in alcoholic solution than in a lanolin solution.

Treatment with smaller doses of testosterone for a shorter period of time in the present experiment had approximately the same effect as the previously reported large doses over a longer period of time. The increase in penis size was about the same whether the hormone was given subcutaneously or applied to the penis. This is in agreement with the idea that although the sex hormones are absorbed percutaneously and do have systemic effects, their maximal effect is at the point of application. Our procedure was too crude, however, to allow definite conclusions as to the relative systemic effectiveness of percutaneous and subcutaneous testosterone.

SUMMARY

The application of 7.5γ testosterone to the penes of castrated immature rats causes a definite growth of this organ in 10 days. This growth is as great as that obtained when the animals are treated with the same daily dose for 22 days. The growth of the penis in rats treated with testosterone in lanolin was less than that obtained with testosterone in alcohol. The daily application of 1γ of estradiol for 22 days or 5γ for 10 days, or the application of 5γ of estrone for 10 days failed to cause any significant increase in the length or weight of the penes of castrate rats.

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EFFECTS OF TESTOSTERONE PROPIONATE ON PREGNANCY AND ON PASSAGE OF OVA THROUGH THE OVIDUCTS OF MICE[1,2]

H. O. BURDICK, BASIL B. EMERSON AND RAE WHITNEY
From the Department of Biology, Alfred University
ALFRED, NEW YORK

SINCE it had been shown that small injections of estrogen begun the day of mating resulted in the retention of fertilized ova in the fallopian tubes (1) and massive doses caused an acceleration of the rate of passage (2), it was thought probable that androgens might also affect the tubal passage of the eggs.

PROCEDURE

To test this possibility, mated mice were selected after the formation of the vaginal plug (a.v.p.) and subcutaneous injections of testosterone propionate were usually begun the day of mating, 6 to 8 hours after the seminal plug had been found.

Over 100 mated animals were used in the experiments. However, those animals autopsied before 70 hours a.v.p. are not reported here because it was found that, in these, the androgen had no apparent effect on either the ova or their tubal passage.

The animals were divided into 5 groups according to the amount of androgen injected. It will be seen in table 1 that the doses varied from 0.025 to 5.0 mg. once daily. It was soon found from preliminary experiments that the smaller doses did not affect tubal passage, while the 5 mg. injections prevented the descent of ova from the oviducts into the uteri. Therefore, intermediate amounts were used to determine the threshold dose of testosterone propionate by which ova could be tube-locked.

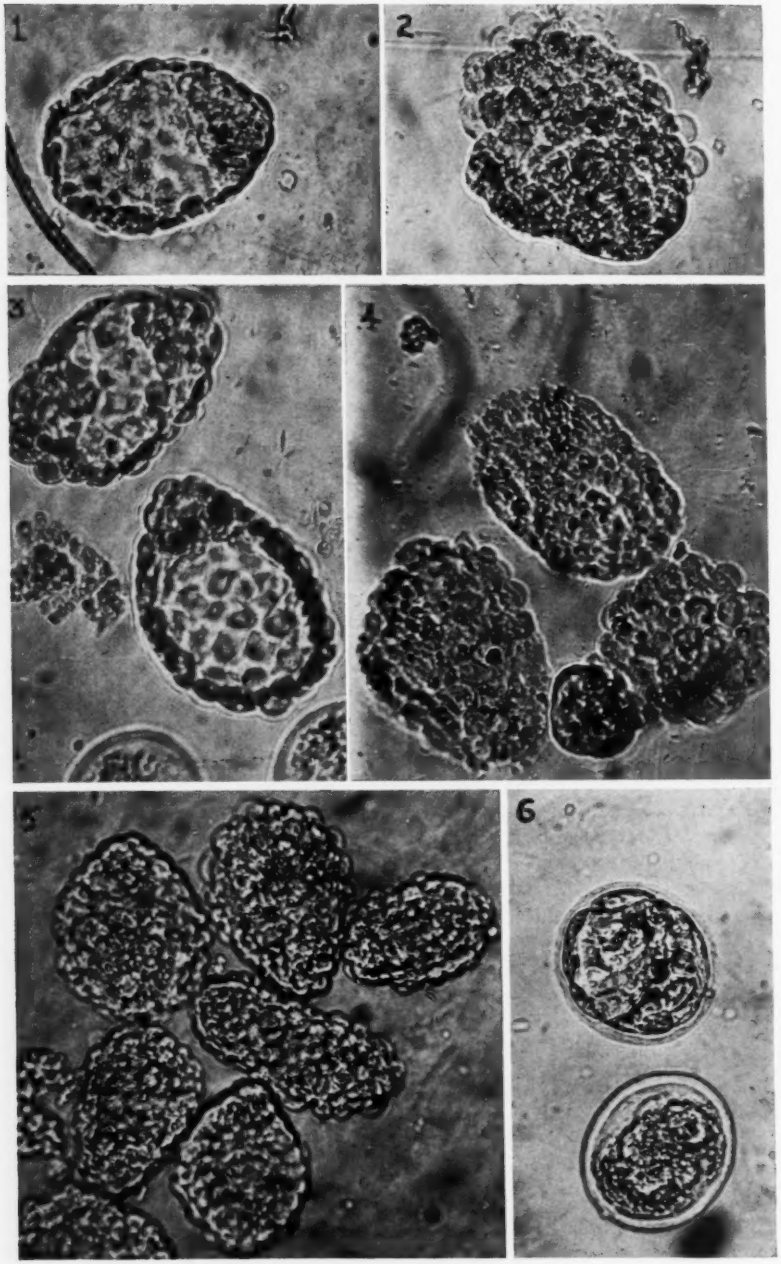
RESULTS

Results with 0.025 mg. testosterone propionate. As indicated above and in table 1, this amount of androgen had no especial effect on the rate of passage, as blastocysts were found in the uterus 85 hours a.v.p. If there were a slight delay in transit, it does not appear in this report because autopsies were performed much later than the time required for normal descent into the uterus.

The blastocysts found after 4 to 6 injections seemed to be perfectly normal in size and color. That both the blastocysts and the uteri were physiologically normal is indicated by the fact that in 4 animals (*115*, *118*, *119*, *140*) actual implants or beaded uteri are reported. The time of implantation is not uniform in these animals. Blastocysts were implanted in animal *119* on the 6th day after 5 injections but were flushed from the uteri on the 6th day in mice *138* and *139* which had received 6 daily injections. Other experiments will have to be performed to determine whether implants can survive more than 5 such injections, inasmuch as the implants appeared abnormal in *mouse 140*, and were absent in animal *120* after 10 daily injections.

[1] These experiments were made possible by a grant from the Penrose Fund of the American Philosophical Society and were administered through the courtesy of Mr. C. F. Randolph, Treasurer of Alfred University.

[2] The testosterone propionate was furnished through the courtesy of Doctors Gregory Stragnell and Max Gilbert of the Schering Corporation and Dr. R. Macbrayer of the Ciba Pharmaceutical Products, Inc.



Figs. 1–6. (See opposite page for explanation).

Results with 0.25 mg. Although this amount did not cause tubal retention, the blastocysts in animals 78 and 83 were all in the ovarian end of the uterus at 84 and 133 hours a.v.p. Evidently the ova were delayed in descent or else the rather quiet uterus had failed, as yet, to conduct the blastocysts to implantation sites. Hemorrhagic areas were noted in the endometrium of *mouse* 76 at 151 hours a.v.p.

Results with 0.5 mg. In this group there was some evidence of retardation in that the eggs of animals 21 and 13 were taken from the tubes at 77 and 79 hours a.v.p. respectively. This is also true of the blastocysts of *mouse* 24 at 99 hours a.v.p. In all other instances, blastocysts were found in the flushes from the uteri. Of especial interest is the fact that in animals 42 (fig. 1) and 43 blastocysts were intact after 6 injections and had not implanted by the 7th day a.v.p. The extreme example of this condition was in *mouse* 44 (fig. 2) with 8 injections and blastocysts free in the uterine lumen on the ninth day, 208 hours a.v.p.

Results with 2.0 mg. In 3 of the 6 animals reported here, some or all of the eggs were flushed from the uterus 85 to 170 hours a.v.p. However, that this amount of testosterone propionate is a threshold dose necessary to tube-lock ova is evident from the fact that in the remaining animals some or all of the eggs were still in the tubes at 87, 127 and 170 hours a.v.p. Figure 3 shows 2 normal blastocysts and 2 degenerate eggs taken from the ovarian third of the left tube 127 hours a.v.p.

Results with 5.0 mg. The blastocysts in these animals were, with but few exceptions, retained in the oviducts. Of the 22 animals reported, in only 3 (29, 30 and 31) had all the eggs gone from the oviducts into the uteri. These 3 animals were selected on the same day and were injected from the same supply of testosterone propionate.

Some of the late blastocysts in this group show signs of deterioration such as herniation or a general collapse. The 0.3% saline solution used in this work may be partly responsible for these pathological changes as we have since found that a 0.1% saline solution will better sustain these blastocysts until photographed.

Of especial interest is the finding of late blastocysts without zonas in the ovarian third of the tubes in animals 37, (fig.4) 38 and 39 (fig. 5) 133 to 149 hours a.v.p. Why they were located in this region is not known unless it might be that the animals were injected just before or soon after ovulation so that peristalsis was completely suppressed before the eggs got into the isthmus.

Effects of testosterone propionate on pregnancy. In a series of 10 animals injected several days in amounts varying from 0.5 mg. to 2 mg., not one showed implants when autopsied 9 to 15 days a.v.p.

DISCUSSION

At the time these experiments were initiated, it was uncertain whether testosterone propionate assisted or inhibited the action of hormones of early pregnancy. Some investigators (3, 4, 5) have reported that testosterone propionate was estrogenic in action since it induced the early opening of the vaginal canal in immature

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BLASTOCYSTS FROM ANIMALS INJECTED WITH TESTOSTERONE PROPIONATE. ×225

Fig. 1. Normal appearing blastocysts found 146 hr. a.v.p. in the uterus of *mouse* 42 which had received six 0.5 mg. injections. Fig. 2. Uterine blastocyst from *mouse* 44 found 208 hr. a.v.p. after 8 injections of 0.5 mg. This blastocyst is collapsed and herniated. Fig. 3. Two normal appearing blastocysts and two degenerate cells with zonas—presumably unfertilized. Blastocysts were obtained from the ovarian third of a tube from *mouse* 33, 127 hr. a.v.p. after 4 injections of 2 mg. Fig. 4. Blastocysts from the ovarian third of a tube from *mouse* 37, 134 hr. a.v.p. after 4 injections of 5 mg. The blastocysts show irregularities in development. Fig. 5. Blastocysts from the ovarian third of oviduct from *mouse* 39, 146 hr. a.v.p. after 3 injections of 5 mg. Fig. 6. Early blastocysts with zonas taken from a tube of *mouse* 26, 86 hr. a.v.p. after 2 injections of 10 mg. One blastocyst was collapsed and yellowish.

TABLE 1

Animal No.	Number of daily injections	Autopsy hr. a.v.p.	Position of eggs T—tube; U—uterus
		Injections: 0.025 mg. daily	
111	4	85	U
113	4	95	U
114	4	96	U
112	5	100	U
116	4	100	U
115	5	101	U (implants?)
117	4	111	U
118	4	112	U (implants?)
138	6	133	U
139	6	133	U
119	5	146	U (implants)
140	6	148	U (implants?)
		Injections: 0.25 mg. daily	
77	3	84	U
83	4	133	U
76	5	151	U (implants?)
		Injections: 0.5 mg. daily	
21	2	77	T
13	3	79	T
24	2	99	T and U
40	5	131	U
41	5	132	U
75	5	133	U
42	6	151	U
43	6	156	U
45	4	158	U
44	8	203	T and U
		Injections: 2.0 mg. daily	
12	2	83	U
23	2	85	T and U
2	3	86	U
33	4	127	T
34	4	133	U
35	5	170	T and U
		Injections: 5.0 mg. daily	
22	2	84	T
29	3	84	U
30	3	84	U
31	3	87	U
31	4	97	T
49	3	107	T
46	3	109	T
47	2	109	T and U
50	4	133	T
38	3	133	T and U (?)
37	4	134	T and U
52	3	135	T
59	5	135	T
60	5	135	T
32	5	146	T and U
39	3	146	T
48	3	156	T
63	3	156	T and U
53	6	159	T and U
58	7	179	T

animals, and caused reddening of the sexual skin in monkeys (6). On the other hand, Salmon, Walter and Geist (7) concluded that testosterone propionate inactivates estrogen in women; Robson (8) proved that testosterone propionate inhibited estrous cycles in mice and Scipiades (9) demonstrated that it has a limited progesterone-like action in pregnant castrated rats. Scipiades also stated that large doses resulted in the death of the embryos, but more recently Greene and Burrill (10) have shown that not only do some of the androgens have a progesterone-like action in rats ovariectomized in the last third of gestation but that, contrary to Scipiades' conclusion, the young can survive only if massive doses of androgens are given.

In these experiments testosterone propionate, in the smaller doses, does not inactivate the estrogen necessary for the tubal passage of eggs but injections of 5 mg. daily result in the tubal retention of ova. The conclusion that this amount overrides or replaces the estrogen necessary for tubal contractions is substantiated by the results of several experiments. Geist, Mintz and Salmon (11) reported that normal tubal peristalsis in women is completely suppressed by adequate dosages of testosterone propionate, while Robson (12), and Leonard, Sager and Hamilton (13) have shown that the typical uterine contractions in estrous rabbits are prevented by this androgen. Furthermore, the uteri were not activated by oxytocic substances, a result which is much like that following progesterone injections.

In considering the effect of testosterone propionate on the developing blastocysts in these experiments, it appears certain that in the higher doses there is either a direct or indirect favorable action because the blastocysts are preserved for some hours even in the oviducts. This is in sharp contrast to the results with 5 to 10 R.U. of estrogen (1) where the tube-locked ova collapsed soon after having attained the blastocyst stage. However, the continued use of testosterone propionate, even in the 0.5 mg. doses, is detrimental to continued embryonic growth.

Since it is now established that testosterone propionate causes the repression and resorption of the corpora lutea of early pregnancy (14), of induced ovulation (15), and of normal corpora lutea (16) it is believed that the early embryos suffer from the lack of estrogens normally secreted by the corpora lutea.

In all these experiments there was no evidence of toxicity shown in the behavior of the animals even with 5 mg. over an 8-day period.

SUMMARY

The experiments reported here show that pregnancy may be interrupted by testosterone propionate, and that adequate daily doses will cause the retention of blastocysts in the oviducts. Since previous experiments have shown that testosterone propionate causes the repression and resorption of the corpora lutea of early pregnancy, it is concluded that, in these experiments, testosterone propionate overrides the peristaltic action of estrogen and finally replaces it. Testosterone propionate has either a direct or indirect progesterone-like action because the eggs continue to develop into apparently normal blastocysts while retained in the tubes.

In mice the daily injection of 0.025 mg. during the first days of pregnancy does not interfere with the descent of ova from the oviducts into the uteri. Blastocysts continue to develop and some implants are reported.

With 0.5 mg. daily, there is some evidence of retardation of tubal passage. Blastocysts were sometimes recovered from the tubes 77 to 99 hours after the vaginal plug. Normal appearing blastocysts may be free in the uterine lumen on the 9th day after mating. No implants were found.

The results show that 2 mg. daily is the liminal amount of testosterone propionate

necessary to cause retention of blastocysts in the tubes beyond 100 hours after the vaginal plug.

Daily injections of 5 mg. do not interfere with blastocyst development, but cause their retention in the tubes where disintegration of the blastocysts finally takes place. This is in contrast to the results found in earlier experiments with 10 R.U. of estrogen, in which the ova disintegrated in the early blastocyst stage. No evidence of toxicity was noted in the behavior of animals even after eight 5 mg. injections.

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UROLITHIASIS IN THE RAT AFTER INJECTION OF TESTOSTERONE PROPIONATE

J. GERSHON-COHEN, HARRY SHAY, KARL E. PASCHKIS AND SAMUEL S. FELS

From the Medical Research Laboratory, Samuel S. Fels Fund

PHILADELPHIA, PENNSYLVANIA

WE HAVE REPORTED recently the occurrence of large cystic ovarian tumors following the injection of large doses of testosterone propionate in Wistar strain rats (1). In many of these rats we also observed the occurrence of urinary calculi. Burns and Schenken have also reported the development of urinary calculi but in female mice of the C_3H strain following the injection of large doses of estradiol benzoate in sesame oil (2).

Wistar strain rats bred in our own colony for 3 years were used in the experiment. Subcutaneous injections of testosterone propionate[1] were started on the first or second day of life. Forty-six female rats were used with litter mates as controls. Owing to the fact that one litter mate sometimes served as control for 2 or 3 sisters, the number of controls totalled 18. However, for the observation reported in this paper every normal female rat of our colony autopsied in the course of other experiments could be considered as a control.

During the first week, 0.5 mg. testosterone propionate was given 3 times, thereafter 1.0 mg., 3 times weekly. Animals were killed from the 15th to the 60th week; in the 14 rats where calculi were found, 9 had a total dosage of 100 mg. or more and 5 had doses varying from 50 to 100 mg.

The diet consisted of Purina Dog Chow with lettuce twice weekly and fresh water, which was constantly available. The presence of calculi in the bladder was determined by roentgen examination in two of the living, but as all the animals were autopsied, this report is based on the necropsy findings only. Although urographic examinations were made in many animals, using 4 cc. of diodrast, its value was not well established due to the faintness of the density of dye in the urinary tract everywhere excepting in the bladder.

Forty-six female rats treated as described above have been examined. In 14 cases stones were found in the urinary tract at autopsy. In none of the 18 litter-mate female control animals and in no other female rat autopsied in the course of various other experiments, in the course of 3 years, was urolithiasis ever found. In 20 male rats treated with testosterone propionate in the same way as the females discussed in this paper, no case of urolithiasis was found. We did, however, once find stones in the renal pelvis of a male rat of our colony which had never been used for an experiment of any kind and which had died spontaneously.

In 12 of the 14 cases stones were present in the bladder varying in number from 1 to 10. In 5 rats the bladder was the only site of stones at autopsy, in the others, stones were also present in the ureters (4 cases) renal pelvis (3 cases) and urethra

[1] Testosterone propionate (oreton) was furnished by the Schering Corporation through the courtesy of Dr. Max Gilbert.

(1 case). Two of the 14 cases presented stones only in the upper urinary tract and not in the bladder.

The sequelae of urolithiasis were marked in most cases. The wall of the bladder was hypertrophied, the ureters dilated and twisted (8 cases), the renal pelvis dilated (5 cases), and the renal tissue atrophied due to hydronephrosis. Infection due to urinary stasis had taken place in some cases with pyelonephritis in two cases and a large abscess of the kidney in a third. Bacteriological cultures of urine were taken in several

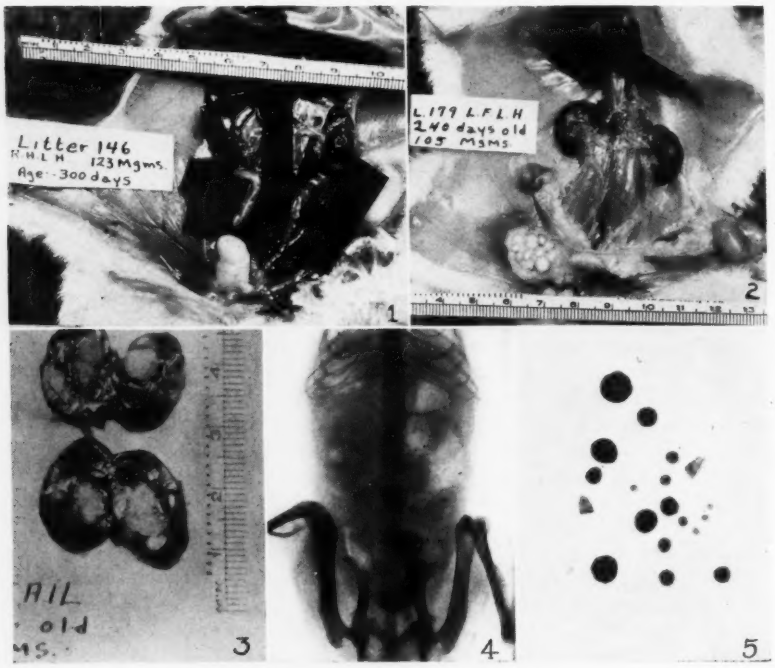

Fig. 1. Female rat (*L 146 rhlh*) received a total dose of 123 mg. testosterone propionate. Bladder filled with stones, unopened. Considerable dilatation of ureters and renal pelvis. Fig. 2. Female rat (*L 179 lfhl*) received a total dose of 105 mg. testosterone propionate. Bladder opened, filled with stones. Diverticulum of the bladder, opened after this photograph was taken, found to contain stones. Dilatation and twisting of right ureter. Fig. 3. Female rat (*L 148 t*) received a total dose of 134 mg. testosterone propionate. Kidneys opened. Both renal pelves are blocked by cast-like stones. Fig. 4. Roentgenograph, female rat (*L 179, LFLH*) showing opaque stones in bladder. Fig. 5. Roentgenograph of stones removed from bladder of female rat (*L 179, LFLH*).

cases, both in broth and on agar, revealing growth of either bacterium coli or bacillus proteus.

We should like to note the finding of a parasite, *trichosoma crassicauda* in the bladder of many of our rats both treated and controls. Murray and Steinkamm (3) have recently reported their findings of this parasite in the rat's bladder. However, it should be emphasized that there is no coincidence of parasites and stone formation in our cases. Parasites were present in some of the control rats having received no treatment whatsoever.

Murray and Steinkamm (3) have described hyperplasia of the epithelium of the

bladder due to this parasite. We cannot confirm this finding. In the control animals bearing the parasite, there was no trace of epithelial hyperplasia. In the testosterone treated animals there is a tendency to hyperplasia of the epithelium of the bladder. This was quite marked in several cases, but it did not coincide with the finding of parasites; neither was the hyperplasia limited to the cases with urolithiasis. Marked hyperplasia was found in testosterone treated rats which were free of stones and free of parasites.

Epithelial growth has been described by Lacassagne (4) in the bladder and urethra of mice treated with estrone. Lacassagne considered this epithelial hyperplasia as causing mechanical obstruction and consequent dilatation of the upper urinary tract. His findings are at variance with those of Burrows (5) in mice and Murray and Steinkamm (3) in rats treated with estrogen. In none of our testosterone treated animals was the moderate epithelial hyperplasia in any way to be compared with the enormous new growth described by Lacassagne and there seemed no possibility to us that the hyperplastic epithelium in our rats produces a mechanical stasis of urine.

Qualitative analysis of the stones, performed in the Chemistry Department of our Laboratory by Dr. F. L. Munro, showed them to be composed of calcium phosphate. Faint traces of sterol were also present, but this can easily be explained by the fact that the animals were receiving large doses of testosterone propionate and were consequently found to excrete sterols. The calculi were soft and gray-white in appearance. They varied in size from gravel to stones 1 to 6 mm. in diameter, often faceted. No explanation for the genesis of the urolithiasis can be offered. Mechanical obstruction of the urethra was considered a possible cause. Development of a prostate in female rats treated with testosterone has been reported. But stones were present in cases in which, not even histologically, was there any trace of prostatic tissue.

We can definitely state that mechanical obstruction of any kind was absent in our cases as revealed by gross and histological examination, the latter in a number of cases including studies of serial sections.

As to the influence of sex hormones on calcium and phosphorus metabolism, results of various authors are still somewhat contradictory. Increased output of calcium with decalcification of bones under the influence of estrone has been reported.

Gardener and Pfeiffer (6), on the other hand, described a hypercalcification under estrogen treatment. This effect was found to be counteracted by simultaneous administration of testosterone. Earlier Marlow and Koch (7) had failed to find any significant changes in blood calcium in fowls following administration of male sex hormone prepared with bull testes. Recently Thorn and Engel (8) have reported a marked decrease in phosphorus excretion in testosterone treated dogs.

We have so far studied the urinary excretion of calcium and phosphorus in 4 of our testosterone treated female rats and their respective untreated litter mates. The rats were put in metabolism cages and urine collected in 24-hour samples. The analyses were performed by Dr. F. L. Munro of the Chemistry Department of our Laboratory. Two of the testosterone treated rats showed stones at autopsy whereas the other 2 revealed a normal urinary tract. Calcium output was considerably higher than that of the control in one stone-bearing animal (treated with 1.75 mg./24 hours; control, 0.33 mg./24 hours), but considerably lower than that of its litter-mate control in the second urolithiasis rat (treated with 0.68 mg./24 hours; control, 1.73 mg./24 hours). In the 2 other testosterone treated rats free of stones, calcium output was lower than that of their untreated litter mates. Phosphorus values showed no significant differences. Hence, we must conclude that so far, we have not found any changes in calcium metabolism which would allow an explanation of the urolithiasis.

Our studies of phosphorus and calcium metabolism on animals under treatment with large doses of testosterone propionate should, however, be considered as preliminary since more extended studies may reveal significant changes.

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LIMITATION OF FOOD CONSUMPTION AS A FACTOR INFLUENCING ENDOCRINE REACTIONS IN THE CHICK

W. R. BRENEMAN[1]

From the Waterman Institute and Zoology Department, Indiana University[2]

BLOOMINGTON, INDIANA

THE EFFECT of inanition on rats has been reported by Mulinos et al. (1) and Pomerantz and Mulinos (2) who suggested that a pseudo-hypophysectomized condition was produced as a result of limiting food consumption. McKay et al. (3, 4, 5) made an extensive study of limitation of diet as a factor which prevents aging in rats; i.e., extends the juvenile condition, and these results also suggest a decrease if not total absence of pituitary secretion. Pituitary activity in both groups of experiments, however, is apparently restored upon resumption of normal diet. Byerly and Burrows (6) reported that chicks given no food or water for as long as 72 or 96 hours after hatching were excellent test animals for pituitary gonadotropin. Gonad weights were lower than in control animals and variability was apparently less.

It was hoped that the inanition technic might be used for a critical study of the effects of androgens on the White Leghorn chick. Work on this form has shown that there is considerable variation in comb size and organ weights, and this variability is a considerable handicap in the use of chicks for the purpose of androgen assay. It was noticed in previous experiments in our laboratory that chicks which received an amount of food inadequate for normal growth seemed to be less variable and organ weights were lower than in controls. This similarity to the results of the Pomerantz and others cited above led to the present study which is an analysis of some endocrine experimentation with chicks given a limited diet.

MATERIAL AND METHODS

A total of 386 White Leghorn chicks was used in this study. All birds were fed a standard starting mash throughout the experimental period. The birds were kept in a battery brooder and one group, designated as 'normal diet' had access to food at all times; the other group, represented as 'limited diet,' was given food only on alternate days, but was permitted to eat all the mash they wanted on feeding days. Body weight in all tables is represented as weight after the removal of the crop and combs are shown by a factor which is the square root of height times length in millimeters ($\sqrt{H \times L}$ mm.) or by weight of the comb which was removed by cutting parallel to the skull with scissors. The androgen used was oreton-B (dihydroandrosterone-benzoate) in sesame oil solution and the estrogen employed was theelin in sesame oil. An unfractionated pyridine extract of whole hog pituitary powder which had been freed of some impurities by 33% acetone treatment (7) was the pituitary hormone injected. The results of the experiments have been summarized in

[1] The author is indebted to Dr. Max Gilbert of the Schering Corporation for supplies of oreton-B and to Dr. Oliver Kamm, Parke, Davis & Co., for the theelin used in these experiments. Technical assistance was given by the N.Y.A.

[2] Contribution No. 86 of the Waterman Institute and No. 284 of the Zoology Department, Indiana University.

tables 1, 2 and 3; the first two of which represent more preliminary analyses of the problem.

RESULTS AND DISCUSSION

The data in table 1 demonstrate the difference in body and testes weights and comb size over a 30-day period in normal and limited diet chicks when limitation of

TABLE 1. LOW DIET AND CONTROL SERIES

Treatment	No. of animals	Weight		Testes: Body wt.	Comb factor	Comb factor: Body wt.
		Body	Testes			
		gm.	mg.	mg.		
		10-day				
Limited diet, food on days 1, 3, 5, 7, 9	14	45.0	8.0	13.4[1]	5.1	8.5[1]
Control	12	79.3	17.0	16.7[1]	6.5	6.1[1]
		20-day				
Limited diet, food on days 1, 3, 5, 7, 9, 11, 13, 15, 17, 19	15	106.0	27.0	38.2[2]	13.4	18.9[2]
Control	13	165.0	53.6	48.7[2]	16.1	14.6[2]
		30-day				
Limited diet, food on days 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, etc.	13	133.0	26.6	50.0[3]	12.8	24.1[3]
Control	15	255.4	72.5	70.9[3]	21.4	20.9[3]

[1] per 75 gm. [2] per 150 gm. [3] per 250 gm.

food intake is the only variable factor. The body weight average of chicks on the limited diet is much lighter than is the control average. It must be emphasized, however, that these birds were not starving. There is yolk present which helps maintain the birds for the first few days after hatching, but after that time they do not show any particular weakness or abnormality and at autopsy there is always fat present in the body cavity. The death rate in the limited diet series is no greater than in the normal, averaging about 2%. Unpublished data, however, show that the removal of food for an additional day will promptly result in a great increase in the death rate.

That the growth rate of chicks on a limited diet is greatly decreased is shown by the body weight average at 30 days, which is approximately 50% of that of the control birds and comb and testes likewise are smaller than those of the normal diet series. Comparison of these data, however, should be made after correction for body weight differences in order to determine if the averages vary in proportion to the body weight. Such a correction is made on the basis of a body weight of 75 gm. on the 10th day, 150 gm. on the 20th day and 250 gm. on the 30th day. After this adjustment in the mean, it is evident that the testis weight of limited diet chicks is not proportional to body weight but is definitely lower than that of the animals on normal diet. Control testes weights, both actual and corrected, are heavier than those in the limited diet series, a fact which indicates that there has been a suppression of pituitary secretion in the birds of the latter group. This confirms the observations of Pomerantz and Mulinos and this interpretation of the testis data will be discussed further during the consideration of the results in table 2.

The mean comb weight in the limited diet birds, in contrast to testes average, proves to be heavier than that observed in the normal diet chicks. This is rather difficult to explain but some suggestions may be advanced which will perhaps account

in part for this phenomenon. Pezard (8), Hardesty (9), and Breneman (10) have pointed out that two factors must be considered as operating in comb growth; one, comb increment is proportional to body growth, i.e., non-hormonal growth and two, increase in size may be a result of hormone action. Evidence was presented by Breneman in the above paper and subsequently (11) that after the 10th day the testes of the White Leghorn cockerel are secreting an androgenic substance. Since the testis weight is less in the limited diet series, it is likely that less male hormone is being secreted. Comb size, however, in these chicks is only slightly below that of the control, 13.4 as compared to 16.1, and exceeds the control after the body weight adjustment is made. This is notable on the 30th day when the corrected comb factor for the limited diet birds is 24.1 and for the normals, 20.9.

The fact that comb size in the limited diet birds exceeds that of the normal diet birds can probably be explained on the basis that comb growth in the former appears to be chiefly non-hormonal. This is illustrated by comparing net body weight increases from the 10th to 20th day, which in the limited diet birds is 135%. Comb increment at this time is 165% and, therefore, the greater portion of the comb increase is obviously non-hormonal. There also is a 300% net increase in testis weight during the same time which should account for sufficient androgen secretion to produce the remaining 30% growth. The body growth of the normal birds during the 10th to 20th day interval is only 108%, but comb increase is nearly 150%, indicating greater male hormone effect. Proportional body weight increment, on the other hand, is enough lower than in the limited diet animals that adjustment for body weight shows the limited diet birds with greater comb sizes.

There is an actual, though very slight, decrease in both testes and comb sizes in the limited diet series during the 20 to 30-day period, but the growth of the body is not great during this interval. The normal animals show an increase of 90 gm. in body weight which, however, is not accompanied by a corresponding increase in either comb size or testis weight. No adequate explanation can be made at present for this peculiarity, but when this observation is coupled with the fact that comb size in the limited diet group is nearly constant during the same period, it is obvious that here again the body weight correction gives a somewhat misleading figure.

Although body weight variation will probably explain most of the size differences noted in comb growth, the possibility exists that the male hormone formed by the testes in the birds with lower food intake may be more efficiently utilized. Byerly and Burrows (6) in their work on chicks kept without food or water discovered that the testis would respond to surprisingly small amounts of gonadotropin. Breneman (12), using their technic, found good responses with pituitary-chlorophyll and pituitary-hemin mixtures whereas these mixtures in larger amounts resulted in only a moderate degree of stimulation in older chicks which were given food and water. These data suggest that there is a better utilization of hormones in animals on limited diet and the experiments in table 2 are designed to determine whether this is true following pituitary and male hormone injection under the diet conditions of these experiments.

The birds were given 20 mg. equivalent of the original whole hog pituitary powder daily for 5 days and a good response was observed in both normal and limited diet series. Comparison of controls in table 1 with the pituitary injected birds shows that net increase in testicular weight average is about 200% in normal diet and nearly 400% in the limited diet group. Testis weights average 38.4 mg. on the limited diet chicks and 47.4 mg. in the normal diet animals. If these averages are adjusted to 75 gm. of body weight, the former average 62.6 mg. and the latter 40.9 mg. A similar result is noted for comb growth in these birds: the comb factors are 6.2 in the limited

diet series and 8.0 in the normal diet group and after body weight correction these appear as 10.1 in the former and 6.8 in the latter. It is obvious, therefore, that the response is proportionately greater in those birds which were kept on a reduced diet, both with respect to testicular response to gonadotropin and in the subsequent comb growth which is stimulated by the secretion of androgen from the animals' own testes. A second possibility is also suggested, namely, that the testes in the limited diet chicks may secrete a greater amount of androgen than the normal diet birds following pituitary treatment. Two series of chicks were injected with the same amount of oreton-B from the 5th to 9th day and comb measurements were taken in order to analyze this point.

Chicks on a limited diet injected with oreton-B have an average comb factor of 6.6 which is 10.3 per 75 gm. of body weight. The normal diet birds have combs of 8.1 which average 8.8 when weight adjustment is made. It is again evident that

TABLE 2. TEN-DAY TREATED SERIES

Treatment	No. of animals	Weight		Testes: Body wt.	Comb factor	Comb factor: Body wt.
		Body	Testes			
		gm.	mg.	mg./75 gm.		
*Limited diet+20 mg. AP, 5th to 9th day	14	46.0	38.4	62.6	6.2	10.1
Normal diet+20 mg. AP, 5th to 9th day	8	87.1	47.4	40.9	8.0	6.8
*Limited diet+0.25 mg. oreton-B, 5th to 9th day	10	48.0	8.3	12.9	6.6	10.3
Normal diet+0.25 mg. oreton-B, 5th to 9th day	12	68.5	11.5	12.5	8.1	8.8

* Received food on days 1, 3, 5, 7, 9.

the response to a given amount of hormone is proportionately greater in the limited diet animals and that the same situation exists after male hormone treatment as is observed when pituitary preparation is injected. On the basis of comparative effect of male hormone in the two groups, it is not necessary to assume that the injection of pituitary hormone causes more male hormone to be secreted by the testes of the limited diet chicks. The greater comb growth observed in this group is interpreted as a result of more efficient utilization of the hormone available.

The chick apparently differs somewhat from the rat in this respect according to the data reported by Pomerantz and Mulinos. They note a 10% decrease in body weight of their animals and show that they can be made to estruate by the injection of 2 to 4 R.U. of theelin. This would indicate, therefore, that response to hormones is less in the rat during conditions of inanition.

It was noted in table 1 that testes weights are lower in the limited diet series and the effect of the male hormone on the testes weight in such experimental animals should be noted also. When male hormone is given chicks on normal diet (table 2) the testes weight is decreased and does not differ significantly from the average in low diet injected birds, 12.5 mg. compared with 12.9 mg. The mean testes average in the low diet series also is essentially the same in both control and injected birds, 13.4 mg. compared with 12.9 mg. The effect of male hormone on the pituitary secretion of gonadotropin has been considered previously (13, 14), and the evidence re-

ported here is in harmony with previous findings indicating that androgen suppresses the secretion of gonadotropic substance from the pituitary. The observation that the testis average remains the same in both injected and control series of limited diet chicks is significant as evidence that the pituitary gland is secreting little if any gonad-stimulating hormone in these animals, hence male hormone can have no antagonistic effect on testis weight in these birds. Werner (15) has considered this problem in the rat and has been led to a similar conclusion. This point will be discussed further in a subsequent publication.

Frank et al. (16) have indicated that lengthening the injection period increased the reliability of the comb growth response to male hormones in the chick. Burrows,

TABLE 3

Treatment	No. of animals	Body wt.	C.V.	Comb wt.	C.V.	Comb factor	Comb weight	Comb factor
10-day series		gm.	%	mg.	%	$\sqrt{H \times L}$	mg. *Per 75 gm.*	
♂ Limited diet control	12	45.2±3.11	7	13.9±2.72	19	5.2	23.0	8.6
♂ Limited diet+0.125 mg. oreton-B, 5th to 9th day	12	42.9±5.69	13	22.5±10.58	47	5.8	39.3	10.1
♂ Normal diet+0.125 mg. oreton-B, 5th to 9th day	12	63.8±5.95	9	35.9±10.68	30	6.4	42.2	7.5
♀ Limited diet control	12	44.9±1.10	2	10.9±1.80	16	5.0	18.2	8.3
♀ Limited diet+0.125 mg. oreton-B, 5th to 9th day	11	44.9±6.39	10	23.3±6.39	27	6.2	38.9	10.3
♀ Normal diet+0.125 mg. oreton-B, 5th to 9th day	12	69.2±10.40	15	31.9±7.15	22	6.8	34.5	7.4
Normal diet capons	23	76.0±7.49	9	— —	—	5.2	—	5.1
15-day series							*per 100 gm.*	
♂ Limited diet control	11	72.7±11.78	16	41.3±15.25	36	6.8	56.8	9.3
♂ Limited diet+0.125 mg. oreton-B, 5th to 14th day	10	68.2±9.14	13	288.0±69.59	24	13.4	422.2	19.6
♂ Normal diet+0.125 mg. oreton-B, 5th to 14th day	13	95.5±10.08	10	314.0±57.34	17	13.5	328.8	14.2
♂ Normal diet control	20	102.4±17.71	17	96.9±17.62	69	10.5	93.6	10.2
♂ Limited diet+25 I.U. theelin, 5th to 14th day	10	65.6±8.14	12	26.8±13.24	49	6.2	40.8	9.4
♀ Limited diet control	14	63.0±8.96	16	37.9±13.70	36	6.4	60.0	10.1
♀ Limited diet+0.125 mg. oreton-B, 5th to 14th day	11	63.0±13.65	21	258.0±83.59	32	12.6	409.5	20.0
♀ Normal diet+0.125 mg. oreton-B, 5th to 14th day	14	87.7±13.03	14	220.0±110.67	50	11.7	250.8	13.4
♀ Normal diet control	15	92.3±13.77	14	52.1±13.54	25	7.8	56.4	8.4
♀ Limited diet+25 I.U. theelin, 5th to 14th day	13	62.6±11.44	18	24.7±8.64	34	5.7	39.4	9.1
Normal diet capons	21	92.0±12.97	14	— —	—	6.5	—	7.1

Byerly and Evans (17) have suggested that although pullet and cockerel are similar in response to androgens, pullets may be more advantageous for assay purposes because of less variation. Breneman (12), on the other hand, reported that the pullet does not respond as well as the cockerel to androgen. The greater response of limited diet chicks to male hormone suggested that it would be worth while to reinvestigate such factors as the length of injection period, relative response of pullet and cockerel, and variability in the chicks which are on limited diet and the comparison of these results with similar data of normal diet birds. The data in table 3 are concerned with these problems. The comb averages in table 3 include in most instances both comb factor and comb weight because the latter is a more dependable criterion of comb stimulation.

Two time series are represented: one, in which injections were made from the 5th to 9th day and the chicks killed on the 10th, the other, in which the hormone was administered from the 5th to 14th day and the birds killed on the 15th. There is, first of all, a marked uniformity in the body weight in the limited diet chicks in the two series. The lowest body weight in the 10-day series is 42.9±5.96 and the

highest 45.2 ± 3.11, and in the 15-day group the lowest is 63.6 ± 11.44 and the highest 72.7 ± 11.78. The weight range in the normal diet birds is greater in each instance than that observed in the low diet animals; but attention should be called to the fact that at 15 days in these experiments the variation in the limited diet series is greater than it is at 10 days. The coefficient of variation for body weight in the series is interesting. The minimum variation in body weight, 2%, occurs in the 10-day-old low diet control females, and the maximum variability, 21%, in the oreton-B injected 15-day-old low diet females. At 10 days of age the limited diet birds are less variable than the normal diet chicks, but on the 15th day the variation is as great in the limited diet chicks as in the normals and even greater in the one female series mentioned above. Obviously in the 15-day series, limitation of the diet does not decrease the variability of the sample with regard to body weight.

Although the body weight data prove to be somewhat disappointing, the effect of male hormone on comb growth is very significant. Discussion of the results will be limited chiefly to comparisons of comb weights as corrected for uniform body weight. The injection of male hormone into the males of the 10-day limited diet series produced a 10% net increase in comb weight over the controls. This is especially significant since the body weight correction for the combs of normal diet birds gives an average which exceeds the limited diet males by only 2.9 mg. The 10-day females present an even more striking picture of comb stimulation. Oreton-B produced a net increment of 110% in the combs of the limited diet females and when body weight corrections were made, the combs of these birds outweighed those of the androgen injected normal diet animals. The 15-day series, moreover, showed much more marked comb stimulation after male hormone treatment than did the 10-day series. The total amount of oreton-B injected was only twice that given in the shorter time series, but comb weights in the limited diet injected males was 640% greater than in the control. The normal diet injected males, in contrast to this, showed an increment of only 250% over the controls. Injection of oreton-B into the limited diet males is, therefore, more than twice as effective when compared with the control animal than it is in the normal diet cockerels and it is even more remarkable that weight correction shows limited diet animals having combs which average higher than those of the normal diet chick, 422.2 mg. to 328.8 mg. The female birds present similar results. The combs of the limited diet injected pullets exceed the controls by 580% and when compared with the normal diet injected females, they are heavier both in actual comb weight, 288.0 mg. to 222.0 mg., and in corrected weight 409.5 mg. to 250.8 mg. Since the increase in the normal diet injected female animals over the controls is 340%, the limited diet pullets are about 70% more efficient in response to oreton-B treatment.

Two conclusions are evident from these results: first, both male and female limited diet chicks are more responsive to male hormone treatment than are the normal diet animals; and second, the longer injection period is the more effective. This is evidenced by the fact that although the hormone given was only twice as much as that given at 10 days, the comb increment increased from 70% at 10 days to 640% at 15 days in the males and from 110% to 580% in the females. This confirms the conclusion of Frank et al. that the longer injection period is more advantageous for androgen assay purposes.

It is notable, upon further analysis of the results, that the normal diet male has a larger comb following male hormone treatment than does the normal diet female; 42.2 mg. compared with 34.5 mg. in the 10-day series and 328.8 mg. compared with 250.8 mg. in the 15-day series. These results are similar to those previously reported by this author (18). It is remarkable, however, that the limited diet birds do not show

this sex difference. Comb growth in the limited diet females given male hormone is practically equal to that in the limited diet males on both the 10th and 15th day, 38.9 mg. compared with 39.3 mg. and 409.5 mg. compared with 422.2 mg. More surprising is the fact previously noted that limited diet females injected with oreton-B exceed, on the 15th day, the normal diet females receiving the same treatment, 409.5 mg. as against 250.8 mg. These observations raise the question as to why the comb of the normal female should be less responsive to androgen treatment than the comb of the normal male and, moreover, why the limited diet female should exceed the normal diet female to such a marked degree. The fact that the combs in the limited diet females also average more than in normal diet males can probably be explained largely on the basis of more efficient utilization of the hormone in the former, but the difference between the two female series is so much greater that some additional factor must also be involved.

It was suggested (18) that an estrogenic substance might be secreted by the pullet ovary and evidence was presented to demonstrate that theelin would inhibit androgen action on the comb of the chick. A similar observation was reported for cockerel and capon by Hoskins and Koch (19). The data in tables 1 and 2 suggest that secretion of pituitary gonadotropic substance is suppressed by the limited diet treatment and it is likely that any estrogen secreted by the ovaries would, therefore, be decreased in amount and such a decrease in estrogen would mean less inhibition of the injected androgens. This interpretation is recognized as necessitating further experimentation. The injection of theelin into both male and female limited diet animals produced combs which weighed 40.8 mg. and 39.4 mg. respectively with corrected comb factors of 9.4 and 9.1. The comb weights in these chicks are less than that observed for their respective controls, but do exceed the capons which have a comb factor of 7.1. This fact further emphasizes the complexity of the problem inasmuch as the comb is definitely inhibited in its growth in both sexes by injection of an estrogen, but still exceeds that of the capon of the same age. This would further imply that the limited diet animals are secreting a small amount of comb growth-promoting substance.

Since comb growth is so marked in the injected animals, it is important to analyze the degree of variation in comb size to determine if there is greater uniformity in the limited diet series. The data show that the least variation in combs of the 10-day chicks, 16%, occurs in the female low diet controls and that the maximum variability, 47%, is in the oreton-B injected low diet males. Normal diet birds at 10 days show intermediate variation. The 15-day series is in marked contrast to the 10-day series because the minimum variations of 17% and 25% are present in normal male injected and normal female control chicks and the maximum variability also occurs in the normal control males. The low diet chicks are, therefore, intermediate in variability in this instance. It must be concluded that as far as variability in comb size is concerned, there is no advantage in the use of the limited diet birds as test animals for comb growth. The advantage in the method, therefore, is demonstrated by the fact that the limited diet chicks respond more efficiently to given amounts of hormone than do the normal diet birds. This greater response is sufficient to offset to considerable extent the variability.

SUMMARY

Limitation of food in the chick results in a suppression of body growth. When food is available only on alternate days previous to the 30th day, the average body size was only about 50% as great as that of control animals which received food daily. Testes were lighter in the limited diet birds than in control chicks, both in actual

weight and also in proportion to body weight. Although male hormone limited the testes growth in normal diet animals, its effect is not observed in the limited diet chicks. Testes weight of the limited diet and normal diet androgen treated birds are not significantly different.

These facts are interpreted as effects of decreased production of pituitary gonadotropic hormones in limited diet animals. Comb sizes are larger in proportion to body size in the limited than in the normal diet birds. This is probably due to non-hormonic growth of the comb and the fact that it is not as sensitive to lowered hormone level as is the testis.

Injection of oreton-B in the limited diet birds produces a greater comb growth than it does in normal diet animals. Injection from the 5th to 9th day in the limited diet series produces a comb growth 50% to 110% greater than in the normal diet chicks. Hormone administration from the 5th to 14th day was proportionately much greater, the limited diet injected birds exceeding the normal diet controls by from 580% to 640%. Although injection in the normal diet animals shows sex difference in response between male and female after androgen treatment, this sex difference is not apparent in the limited diet series. This is interpreted as indicating the presence of some inhibitor as an estrogen. A study of variability in the series indicates that the limited diet chicks are nearly as variable as the control birds. For androgen assay purposes it is concluded that in spite of this variability the use of limited diet chicks will give a much better test for androgen because of the greater response of the comb in this series. This work also confirms that published from other laboratories with respect to length of injection. The longer injection period of 5 to 14 days proved to be much more efficient in testing on androgen.

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ASSOCIATION NOTICE

SCIENTIFIC PROGRAM OF THE TWENTY-FOURTH ANNUAL MEETING

The Hotel Roosevelt, New York City

MONDAY, JUNE 10

9:30 A.M.

Assay of Adrenotropic Activity Using 2-day Chicks. ROBERT W. BATES, OSCAR RIDDLE and RICHARD A. MILLER, *Cold Spring Harbor, New York.*

Experimental Study of the Similarities Between Cortin and Some Cardiac Glucosides. RAYMUND L. ZWEMER, *New York City.*

Effect of Desoxycorticosterone Acetate on Diffusion of Chloride into the Peritoneum. A. CANTAROW and A. E. RAKOFF, *Philadelphia, Pennsylvania.*

Effect of Adrenal Factors on Plasma Proteins. FRANK A. HARTMAN, LENA A. LEWIS, JONATHAN S. THATCHER and HAROLD R. STREET, *Columbus, Ohio.*

Adrenal Cortex and Carbohydrate Metabolism. GEORGE W. THORN, GEORGE F. KOEPF, ROGER A. LEWIS and CARLOS A. DELBUE, *Baltimore, Maryland.*

Some Pitfalls in the Treatment of Addison's Disease. THOMAS H. MCGAVACK, *New York City.*

Treatment of Addison's Disease with Desoxycorticosterone Acetate. W. O. THOMPSON, P. K. THOMPSON, W. S. HOFFMAN and S. G. TAYLOR, III, *Chicago, Illinois.*

Excretion of Cortin After Surgical Operations. P. G. WEIL and J. S. L. BROWNE, *Montreal, Canada.*

Addison's Disease: A Study of the Lesions in 30 Cases. R. W. CRAGG, *Rochester, Minnesota.*

Urinary Androgen Excretion in Relation to Gonadal and Cortico-Adrenal Function. HARRY B. FRIEDGOOD and HELEN L. WHIDDEN, *Boston, Massachusetts.*

2:00 P.M.

Ability of the Liver to Retain Glycogen in Diabetes Mellitus. I. ARTHUR MIRSKY, MORTON KORENBERG and NORTON NELSON, *Cincinnati, Ohio.*

Observations on "Permanent Pituitary Diabetes" in Dogs. F. C. DOHAN and C. A. FISH, *Philadelphia, Pennsylvania.*

Prevention of Diabetes Mellitus. B. A. WATSON, *Minneapolis, Minnesota.*

Dihydrotachysterol (A.T. 10) and Mineral Metabolism: A Metabolic Study. FREDERICK C. WEBER, JR. and HENRY B. RICHARDSON, *New York City.*

Relation of Hypothyroid States to Gastric Motility. FRANK E. HAMILTON, RODGER E. MACQUIGG and GEORGE M. CURTIS, *Columbus, Ohio.*

Sex Hormone Changes Associated with Liver Disease. SAMUEL J. GLASS, HUGH A. EDMONDSON and SYDNEY N. SOLL, *Los Angeles, California.*

Growth and Development of Successive Generations of Albino Rats Under Thymus Treatment. ALBERT SEGALOFF and WARREN O. NELSON, *Detroit, Michigan.*

Hemoconcentration Accompanied by Low Serum Calcium in Vagotomized Parathyroidectomized Dogs. CARL PFEIFFER and CHARLES C. ROBY, *Chicago, Illinois.*

Behavior and Endocrine Regulators of the Internal Environment. CURT P. RICHTER, *Baltimore, Maryland.*

Eunuchoidism. M. A. GOLDZIEHER and S. M. ADLER, *New York City.*

7:00 P.M.

Annual Dinner

President's Address. PHILIP E. SMITH, Professor of Anatomy, College of Physicians and Surgeons, Columbia University, *New York City.*

Presentation of E. R. Squibb & Sons Award for 1940.

TUESDAY, JUNE 11

9:00 A.M.

Effect of Certain Sex Hormones on the Rabbit's Resistance to Infection with Vaccinia. DOUGLAS H. SPRUNT, *Durham, North Carolina.*

Relation of Endocrine Function to Resistance and Immunity. OLIVER COPE and ISRAEL KAPNICK, *Boston, Massachusetts.*

Estrin Content of the Follicular Fluid and Urine of the Mare and Its Relation to Phenomena of the Estrual Cycle. DENNIS T. MAYER, FREDERICK N. ANDREWS and FRED F. MCKENZIE, *Columbia, Missouri.*

Biological Properties of Highly Purified Mare Gonadotropic Hormone. H. H. COLE, R. I. PENCHARZ and H. GOSS, *Davis, California.*

Effects of Equine Gonadotropin in Anovulatory Sterility of Women. BORIS B. RUBENSTEIN, *Cleveland, Ohio.*

Effect of Progesterone and Gonadotropic Hormone of Mare's Serum on Excretion of Estrogens, Androgens and Sodium Pregnandiol Glucuronidate. NORA WINTHER and LEO T. SAMUELS, *Minneapolis, Minnesota.*

Effects of Chorionic Gonadotropin on Height and Osseous Development. GEORGE B. DORFF, *New York City.*

Effect of Testosterone Propionate on Bone Growth and Skeletal Maturation of Normal and Castrated Male Rats. HENRY H. TURNER, ARTHUR A. HELLBAUM and ERNEST LACHMANN, *Oklahoma City, Oklahoma.*

Effect of Estradiol Benzoate and Testosterone Propionate on Disordered Calcium, Phosphorous and Nitrogen Metabolisms in Two Cases of Cushing's Syndrome without Adrenal Tumors. FULLER ALBRIGHT, ESTHER BLOOMBERG and WILLIAM PARSON-*Boston, Massachusetts.*

Effect of Administration of Testosterone Propionate on Androgen Output of Normal and Schizophrenic Subjects. KOSEPH M. LOONEY and MARION G. HOWE, *Worcester, Massachusetts.*

Implantation of Testosterone Compounds in Cases of Male Eunuchoidism. GERSON R. BISKIND, ROBERTO ESCAMILLA and H. LISSER, *San Francisco, California.*

2:00 P.M.

Inhibition of Mammary Growth by Large Amounts of Estrogen. W. U. GARDNER, *New Haven, Connecticut.*

Stilbestrol in the Substitution Treatment of Ovarian Deficiency. E. VON HAAM M. A. HAMMEL, T. E. RARDIN and R. H. SCHOENE, *Columbus, Ohio.*

Alteration of Urinary Excretion of Androgens by Estrogenic Therapy. E. C. HAMBLEN, C. J. PATTEE and W. KENNETH CUYLER, *Durham, North Carolina.*

Daily Urinary Estrogens in Four Normal and Four Abnormal Women. MAURICE V. KAHLER and CARL HABICH, *Indianapolis, Indiana.*

Lack of Inhibition of A P Gonadotropic Secretion by Physiologic Doses of Estradiol. CARL G. HELLER, EMILY J. HELLER and ELMER L. SEVERINGHAUS, *Madison, Wisconsin.*

Physiologic Effects of Implantation of Estrogens in Menopausal and Castrated Women. U. J. SALMON, S. H. GEIST and R. I. WALTER, *New York City.*

Stilbestrol. A Clinical Study. J. P. PRATT, *Detroit, Michigan.*

A Study of Untreated "Fröhlich's Syndrome" without Brain Tumor. SIDNEY C. WERNER, *New York City.*

Use of Sodium Chloride in the Treatment of Hypopituitarism. D. J. STEPHENS, *Rochester, New York.*

Diagnosis and Therapy of Hormonal Changes Secondary to Pituitary Disease. EPHRAIM SHORR, H. E. HARRISON, B. F. STIMMEL and R. S. HOTCHKISS, *New York City.*

5:00 P.M.

Annual Business Meeting

PAPERS TO BE READ BY TITLE

Control of Gonadal Development. L. G. ROWNTREE and N. H. EINHORN, *Philadelphia, Pennsylvania.*

Use of Thyroid Substances in Certain Gastro-Intestinal Disturbances. ROBERT C. MOEHLIG, *Detroit, Michigan.*

Fate of Ovaries which Have Been Grafted in the Ear for Long Periods of Time. R. T. HILL, *Bloomington, Indiana.*

Pancreatic Extracts: Pharmacology and Possible Clinical Application. JOSEPH B. WOLFFE, *Philadelphia, Pennsylvania.*

Species Differences in the Cholinergic Action of Estrogen. SAMUEL R. M. REYNOLDS and FRANCES I. FOSTER, *Brooklyn, New York.*

Gonad-Pituitary Relationship. ARTHUR A. HELLBAUM, *Oklahoma City, Oklahoma.*

Calorigenic Effect and Stimulation of Heart Rate Produced by Thyroid Preparations. ARTHUR E. MEYER, M. M. THOMPSON and H. J. DANOW, *Brooklyn, New York.*

Effect of Lumbar Puncture and a Diuretic Property of the Withdrawn Spinal Fluid in a Case of Diabetes Insipidus. J. J. ESPOSITO and BENJAMIN JABLONS, *New York City.*

Inheritance of Low Glucose Tolerance in Rats. VERSA V. COLE, BEN K. HARNED and CLYDE E. KEELER. *Philadelphia, Pennsylvania.*

Nature of Adrenal Inhibition in Rats Treated with Testosterone Propionate. KARL E. PASCHKIS, HARRY SHAY, J. GERSHON-COHEN and SAMUEL S. FELS, *Philadelphia, Pennsylvania.*

The "Frog" Test (*Xenopus laevis*) for Pregnancy. ABNER I. WEISMAN and CHRISTOPHER W. COATES, *New York City.*

The Rôle of Endocrinology in Voice Production. LEON FELDERMAN, *Philadelphia, Pennsylvania.*

"True" Hermaphroditism—Case Presentation. BERNARD SELIGMAN, SAMUEL KRAUSHAR and CHARLES S. BYRON, *Brooklyn, New York.*

Effect of Testosterone Propionate on Growth. H. S. RUBENSTEIN, *Baltimore, Maryland.*

Effect of High Doses of Theelin upon the Formation of Antibodies in Lobar Pneumonia. E. VON HAMM, M. A. HAMMEL and I. ROSENFELD, *Columbus, Ohio.*

Failure of Stilbestrol to Inhibit or Suppress Lactation in the Human. A. R. ABARBANEL, *New York City.*

Male Hormone in Impotence. HARRY BENJAMIN, *New York City.*

Chemical Determination of Sex Hormones in Complex Solutions. CLARA M. SZEGO and LEO T. SAMUELS, *Minneapolis, Minnesota.*

Critical Survey of 125 Cases of Menstrual Disorders. Their Endocrine Correlation and Therapy. H. I. SEGAL, F. R. SCHECHTER, N. H. COLTON and N. PASTOR, *Philadelphia, Pennsylvania.*

Effect of Simultaneous Estrogen-Progesterone Administration on Vaginal Smear and Epithelium. EPHRAIM SHORR. *New York City.*

Hormonal Production of Lipemia in Birds and Mammals. OSCAR RIDDLE, TELLEF SENUM and DAVID F. OPDYKE, *Cold Spring Harbor, New York.*

Cancer Endocrinologically Considered. R. ALEXANDER BATE, *Louisville, Kentucky.*

CURRENT ENDOCRINE LITERATURE

HANS O. HATERIUS, ABSTRACT EDITOR

Collaborators

Fuller Albright
Wayne J. Atwell
Geoffrey Bourne
Israel Bram
John C. Burch
H. J. Deuel, Jr.
John C. Donaldson
Maurice H. Friedman
Murray B. Gordon
Authur Grollman
Frank A. Hartman
E. C. Hamblen
F. C. Koch
Edward Larson
J. T. Lewis
Joseph M. Looney
A. E. Meyer
Warren O. Nelson
J. P. Pratt
A. T. Rasmussen
Elmer L. Sevringhaus
L. B. Shpiner
D. L. Thomson
R. L. Zwemer

ADVANCE ABSTRACTS

ATKINSON, D. W. A review of experimental and clinical trials of stilbestrol. *To appear in this Journal.*

A brief review is presented of the work of Dodds and others on derivatives of the stilbenes which have an estrogenic activity. Diethylstilbestrol, or stilbestrol, is 3 or 4 times more potent than estrone and has the advantage of being effective orally, parenterally or by inunction. By mouth it loses approximately 50% of its activity, in contrast to 95% in the natural estrogens. There are unpleasant side effects which are thought to be due to the dihydroxydiphenyl component. These are undoubtedly of central origin but are minimal with conservative dosage, which varies from 1 to 5 mg. daily. The current literature is reviewed with emphasis on the experimental and clinical work which shows the similarity of action of these synthetic drugs to natural estrogens.

BISCHOFF, F. Nonspecific augmentation with a highly purified follicle stimulating hormone fraction (Evans). *To appear in this Journal.*

An unfractionated pituitary gonadotropic hormone preparation was not appreciably absorbed by added $Zn(OH)_2$ and did not manifest nonspecific augmentation in the presence of such added $Zn(OH)_2$. The hormone is quantitatively precipitated by first adding the Zn as the sulfate and then precipitating the Zn as hydroxide. Under these conditions marked nonspecific augmentation is produced. A highly purified FSH fraction, prepared in the laboratory of H. M. Evans, produced augmentation (150 to 550%) when administered with Zn or Cu under described conditions. This FSH preparation when given intraperitoneally with a simultaneous subcutaneous injection of the same preparation in Cu combination, gave rise to the antagonist phenomenon, vis., the preparation, by a rapid resorption rate, was able to antagonize its own influence when acting under a delayed resorption rate. In spite of all efforts at purification, the multiple hormone effects can still be produced by the so-called FSH fraction.

CLEVELAND, R. Observations on the chromatin in the nuclei of endometrium from monkeys under experimental conditions. *To appear in this Journal.*

Twelve castrate monkeys were treated with estrogen (progynon-B) and progesterone (proluton), and endometrial biopsies secured before and after treatment. Uniform dosages of 500 I.U. estrogen (100 R.U.) were administered for 14, 21, 28, 35 and 42 days to 2 animals in each of the groups so treated. Two groups of 2 animals each, primed for 14 days with 500 I.U. estrogen daily, received simultaneously 500 I.U. estrogen and 0.80 and 1.6 mg. progesterone (1 and 2 RB.U.) respectively for 14 days. Tissues were fixed in Bouin's fluid and stained with hematoxylin and eosin. The nuclei of the tissues from animals injected with estrogen, regardless of duration of treatment in the experiment, were characterized by their larger size as compared with nuclei in endometrium of castrate monkeys, a relatively thicker nuclear membrane, prominent nucleoli, large clumps of chromatin at some of the intersections of the coarse chromatin network, and wide spaces between the strands of the network. These nuclei were similar in their characteristics to those observed in the proliferative type of human endometrium. Treatment with estrogen and progesterone produced modifications in nuclear structure in some but not in all cells. These modified nuclei were characterized by a thin nuclear membrane, small nucleoli,

and a fine close-meshed chromatin network concealing most of the nucleoplasm. The unmodified nuclei resembled those in the tissues from animals injected with estrogen.

CONSOLAZIO, W. V., AND J. H. TALBOTT. The extraction and determination of 17-ketosteroids in urine. *To appear in this Journal.*

A modification of current methods for extraction of 17-ketosteroids from urine is described. Five hundred cc. of urine are hydrolyzed by steam for 20 minutes. Extraction is performed in a continuous extractor which comprises an extraction column, a conduit for the solvent vapor and an automatic collecting tube for returning the extract to the flask. The extractor is illustrated and described in the original article. Carbon tetrachloride is used as the solvent and the extraction is complete within 60 minutes. The use of ether is eliminated. Recoveries by this method are approximately 100%. From 12 to 16 complete analyses may be performed by one person in one day with 2 units.

CREEVY, C. D., AND C. E. REA. The treatment of impotence by male sex hormone. *To appear in this Journal.*

Twelve patients with impotence were treated by means of testosterone propionate. While these patients felt better psychically following the treatment, there was no improvement in the impotence. A similar result was obtained when sesame oil without testosterone propionate was injected into three patients. It would seem that the benefits of androgenic therapy in the impotent male without hypogonadism are chiefly psychic.

CUYLER, W. K., C. ASHLEY AND E. C. HAMBLEN. Urinary excretion of pregnandiol-complex by males. III Following intramuscular administration of desoxycorticosterone acetate. *To appear in this Journal.*

A healthy male excreted 29 mg. and 16 mg. of sodium pregnandiol glucuronide estimated by Venning's method, after he had received intramuscularly a total of 25 mg. and 50 mg., respectively, of desoxycorticosterone acetate.

DEMPSEY, E. W., AND U. U. UOTILA. The effect of pituitary stalk section upon reproductive phenomena in the female rat. *To appear in this Journal.*

The reproductive cycle, pregnancy and lactation have been studied in rats after section of the pituitary stalk. Following the operation the cycle, ovulation, pregnancy and lactation may proceed normally when the animals are maintained at normal room temperature. The reproductive cycle is lengthened and the periods of cornification are prolonged in normal animals exposed to cold. This depressing action of cold upon the cycle is abolished by pituitary stalk section. Hypophyseal deficiency sometimes occurs after pituitary stalk section. It appears to be a result of inadvertent damage to the anterior pituitary or to its blood supply, and does not represent a physiological effect of denervation.

GREENE, R. R., M. W. BURRILL AND D. M. THOMSON. Further studies on the androgenicity of progesterone. *To appear in this Journal.*

Immature castrate male rats (29 days old) were treated with 2.0 mg. crystalline progesterone daily in oil solution. Treatment was begun immediately after castration and continued for 10 days. This dosage, when administered subcutaneously, maintained the weights of the ventral prostate and seminal vesicles above the untreated castrate level. The same dose, when administered intraperitoneally, was less effective in maintaining the accessories. The prostates of the subcutaneously treated animals showed definite histological evidence of androgenic stimulation, while those of the intraperitoneally treated animals showed little or no histological evidence of stimulation. It is concluded that progesterone, like estrogens and androgens, is more effective when administered subcutaneously than when administered intraperitoneally. Evidence is presented which indicates that progesterone is androgenic per se, or at least that the adrenals are not involved in the androgenic activity exhibited by progesterone.

HARTMAN, F. A., L. A. LEWIS, J. E. GABRIEL, H. J. SPOOR AND K. A. BROWNELL. The effect of cortin and the Na factor on adrenalectomized animals. *To appear in this Journal.*

Adrenalectomized cats have been maintained in good condition by cortin alone for more than 4 months. The plasma Na in these cats was maintained at the low level characteristic of adrenal insufficiency. Adrenal insufficient cats could be returned to normal appetite and behavior by means of cortin alone. Adrenalectomized cats could be kept alive for considerable periods on Na factor alone. Although they rarely lost weight they did not appear normal in their reactions. Adrenalectomized cats treated with desoxycorticosterone acetate appeared similar to those treated with Na factor. The plasma Na level in adrenalectomized cats was determined by the amount of Na factor injected. However when no cortin was present more Na factor was required to give the same response. A rise in plasma K did not seem to be due to lack of a specific factor but rather to insufficiency resulting from inadequacy of both factors. In other words absence of one factor had little effect if the other factor was adequate. Blood urea in the treated adrenalectomized cats was somewhat higher than normal. Those treated

with Na factor alone were usually higher than those treated with cortin alone. Nothing remarkable was observed in the blood sugar under treatment with different adrenal factors. This was also true in the erythrocyte volume per cent. Similar results were obtained in adrenalectomized dogs. It is concluded that the Na factor controls plasma Na but has no direct effect on plasma K, blood urea or blood sugar. Cortin has no effect on Na metabolism except by enabling the Na factor to be more effective. Cortin maintains adrenalectomized animals in good condition at low plasma Na levels without the Na factor.

INGLE, D. J. The effect of two cortin-like compounds upon the body weight and work performance of adrenalectomized rats. *To appear in this Journal.*

Desoxycorticosterone is the most active of the known steroid compounds in its life maintaining capacity. The compound 17-oxy-11-dehydrocorticosterone has little or no life maintenance effect. The earlier observation has been confirmed that 17-hydroxy-11-dehydrocorticosterone has an apparently greater effect upon the work performance of adrenalectomized rats than does desoxycorticosterone when the first 24 hours following operation are used as a test period. In addition, adrenalectomized male rats having an initial body weight of 180 gm. were treated for one week with these two compounds and at the end of this period their work capacity was studied. The rats treated with desoxycorticosterone gained in weight even when the doses were small, and although their capacity to work was improved over that of untreated animals, their performance was poor even when as much as 10 mg. was administered daily. All of the animals treated with 17-hydroxy-11-dehydrocorticosterone lost weight to the same extent as untreated animals, but their work performance was improved above that of the animals treated with desoxycorticosterone. Similar animals treated with a mixture of desoxycorticosterone and 17-hydroxy-11-dehydrocorticosterone performed more work than animals treated with an equal quantity of the single substance. The findings are in harmony with the hypothesis that there are qualitative differences in the biologic effects of some of the cortin-like compounds.

LAMPORT, H. Periodic changes in blood estrogen. *To appear in this Journal.*

A theory, proposed by various authors since 1930, and called by one the push-pull theory, has been put into mathematical form. They have used it to explain the periodic fluctuations in estrogenic hormone in the female, associated with the sexual cycle, in terms of the interrelationship between ovaries and anterior pituitary. Fundamentally, it rests on the facts that estrogen production by the ovaries is dependent on the amount of circulating 'effective' gonadotropic hormone secreted by the pituitary, and that estrogen causes the pituitary to decrease the secretion into the blood of 'effective' gonadotropic hormone. Thus, increasing blood estrogen decreases the amount of circulating 'effective' gonadotropic hormone so that the ovaries produce progressively less estrogen and its blood concentration starts to fall. As a consequence, 'effective' gonadotropic hormone starts to rise, increasing once again blood estrogen so that the cycle is repeated. Mathematical analysis of this hypothesis then leads to the conclusion that it can be correct only if the quantities involved lead to a blood estrogen-time curve known as under-damped harmonic motion. When this possibility is explored, with all doubts concerning the necessary quantities resolved in favor of the hypothesis, it is found completely inadequate. If the duration of the cycle is chosen to be 28 days, the push-pull theory leads to a curve for blood estrogen in relation to time which is practically unperiodic, so small are its fluctuations. Some other cause not considered in the hypothesis must be sought.

LOEHNER, C. A. Further observations on the use of adrenal cortex extract in the psychotic and nonpsychotic patient. *To appear in this Journal.*

The author reports on 14 mental, medical and surgical patients who have been treated with adrenal cortex extract. He found no basis for the statement that cortex extract may injure the adrenal glands as reported by Ingle, but believes that small doses are definitely beneficial in corticoadrenal insufficiency. The therapeutic use of a small dose of extract is advocated in lieu of involved tests. In mental diseases the psychoneurotic, dementia praecox, and manic patients were apparently benefited. The true depressions have not responded to this therapy. Clinical experience with this method apparently corroborates the work of Liddell and his associates who produced neuroses in sheep. The apparent relationship between corticoadrenal insufficiency and mental disease is again pointed out, and a possible mechanism for the development of mental disease is offered.

LOEWENBERG, S. A., AND N. G. SLOANE. Diabetes insipidus of over 20 years' standing improved following a subarachnoid hemorrhage. *To appear in this Journal.*

A case of diabetes insipidus with moderate hypertension of approximately 20 years' duration that has improved in both the diabetes insipidus symptoms, and in the hypertension, following a spontaneous subarachnoid hemorrhage

MARLOW, H. W., AND D. RICHERT. Avian estrogens and blood calcium. *To appear in this Journal.*

The introduction of many times the amount of

estrogens that is contained in the fowl's body is far below the amount of the pure estrogens which has to be injected in order to change blood calcium level. It is still the opinion of the authors that the raising of the blood calcium by ovarian hormones has not been proved as the sole factor, or even the main factor in such a phenomena, in amounts normally present in an animal.

PERLA, D., AND J. MARMORSTON. Suprarenal cortical hormone and salt in the treatment of pneumonia and other severe infections. *To appear in this Journal.*

The value of parenterally administered suprarenal cortical hormone (together with salt and excess fluid by mouth) was demonstrated in a number of severe infections, including 17 cases of pneumonia, a case of induced malaria with periods of collapse and in severe grippal infections. The criteria of its value were noted in the maintenance of normal blood pressure, decrease in signs of toxicity, avoidance of circulatory collapse, maintenance of appetite and sense of well-being, decrease in the tendency to complications, absence of distention and shortening of convalescence.

ROSS, E. S., AND F. C. MCLEAN. The influence of the growth promoting hormone of the anterior lobe of the pituitary gland upon growth activity in the long bones of the rat. *To appear in this Journal.*

A preparation of the growth promoting hormone of the anterior lobe of the pituitary was administered subcutaneously daily for 7 to 12 days to 9 female and 3 male rats after the weight plateau was established at the age of 6 months or shortly thereafter. All of the treated animals responded by a marked increase in activity of the growth apparatus, including the epiphyseal cartilage plate and the adjacent spongiosa, which was intact but quiescent in the control animals. Three of the treated animals, including 2 of the 3 males, failed to respond by an increase of at least 4% of the body weight. Criteria for the rating of activity in the growth apparatus of the long bones are described. Intraperitoneal administration of two preparations of the growth promoting hormone failed to give consistent results with respect to increase either in growth activity or in body weight. In no case, following administration of the growth promoting hormone, was there resumption of endochondral ossification in the articular cartilage.

RUBENSTEIN, B. B. The vaginal smear—basal body temperature technic and its application to the study of functional sterility in women. *To appear in this Journal.*

The vaginal smears of the various phases of the menstrual cycle in normal women are described in detail. It is shown that the cell types and smear varieties are merely stages of a continuous cycle of growth (under the influence of estrogen) followed by degeneration and desquamation (accelerated by progesterone and estrogen withdrawal). The basal-body temperature cycle is described. The preovulative low temperatures occur together with the proliferative cornified smears, during the phase of follicle development; the ovulative-post ovulative steep temperature rise (more than 1.2°F.) is associated with smears showing marked desquamation and aggregation of cornified cells. When ovulation fails to occur the "ovulative" temperature rise is gradual and usually less than 1.0°F.; the vaginal smear change is also gradual. Conception by coitus at the time of low temperature—cornified smear fails to occur unless the sudden change in smear and temperature usually associated with ovulation also occurs. The application of this technic in functional sterility offers a method for evaluating the patients' gonad function and for assessing the effectiveness of equine gonadotrophin (gonadogen, Upjohn) in inducing ovulation. By use of the diagnostic and therapeutic aid thus available, more than 50% of a group of 48 functional sterility patients were enabled to conceive.

SONDERN, C. W., AND J. L. SEALEY. A critical study of the comparative estrogenic potency of diethylstilbestrol, estrone, estradiol and estriol by oral and subcutaneous administration to mice and rats. *To appear in this Journal.*

The comparative estrogenic activity of stilbestrol, estrone, estradiol and estriol by oral and subcutaneous administration has been presented. (1) The oral administration of graded doses of stilbestrol to rats and mice resulted in a response almost identical to that obtained by Coward and Burn and D'Amour and Gustavson for natural estrogens using 50% response as unity. (2) Assayed orally in spayed adult mice the estrogenic effect of stilbestrol was 10 times estriol, 20 times estradiol and 65 times estrone; assayed orally in rats its effect was 30 times estriol, 65 times estradiol and 80 times estrone. (3) When assayed on spayed adult mice and rats by subcutaneous injection stilbestrol was found to have about the same order of potency as estrone. (4) Comparison of stilbestrol oral with estrone injected indicates that stilbestrol is 25% as effective in mice and approximately equally effective in rats.

TUM SUDEN, C., AND L. C. WYMAN. Adrenalin-induced opacities of the lens in the rat. *To appear in this Journal.*

The rat was found to be at least twice as susceptible to adrenalin intoxication as the mouse. Death or recovery ensued so rapidly after treatment that, unlike the mouse, there was not sufficient time for the development of lenticular opacities. The same was true of adrenalectomized rats with transplants of cortical tissue. In adrena-

lectomized rats, and in normal rats which had been pretreated with histamine or potassium chloride, the acute reaction to adrenalin poisoning was reduced in intensity, thus increasing the survival time as well as the survival rate. In such animals transitory opacities of the lens were obtainable following the injection of the adrenalin. Pretreatment of adrenalectomized rats with ergotamine prevented opacification of the lens, but manometric studies of blood pressure showed that the process did not depend upon hypertension. Blood sugar determinations made at various stages in rats from each experimental group showed that a relativeyl low blood sugar accompanied the formation of cataract but there was an increase in non-glucose reducing substances. It was concluded that the process of opacification of the lens was related to a derangement of carbohydrate metabolism (other than simple hyperglycemia) associated with conditions favoring circulatory collapse, anhydremia and tissue edema. It is suggested that depletion or failure of cortico-adrenal activity may underlie the systemic changes necessary for adrenalin-induced cataract in both rats and mice.

WATSON, E. H., AND R. C. MOEHLIG. Suggestions for conducting growth studies. *To appear in this Journal.*

Investigation and continued study of growth retardation cases are most interesting and fruitful when carefully planned. A method of approach to the diagnosis and continued study of these cases is outlined. Factors which influence growth include heredity, nutrition, past and present health, and endocrine status. Findings which are sigficant in the diagnosis of etiology are enumerated, and illustrations of several of the more common endocrine types are shown. The article, written with the general practitioner in mind, was stimulated by the relative paucity of material of a similar nature in the medical literature and the very keen interest of all physicians in the growth of children, particularly its endocrine aspects.

ABSTRACTS OF ENDOCRINE LITERATURE

ADRENALS

WADA, M., K. FUZIL, H. SIBUTA, AND H. SAKURAI. The action of histamine upon the epinephrine secretion from the suprarenal gland in non-anaesthetized dogs. *Tohoku J. Exper. Med.* 37: 442. 1940.

The effect of histamine administration upon the rate of output of epinephrin of non-anaesthetized dogs was studied by sampling the suprarenal vein blood. The concentration of epinephrin was measured by the rabbit intestine segment method and that of blood sugar by the method of Fujita and Iwatake. Histamine was injected intravenously in a dose of 1, 2, 3, and 5 mg. per kg. The rate of output of epinephrin was increased from 0.00002 to 0.00003 mg. per kg. per minute to 0.0003 to 0.0007 mg. per kg. per minute for 1 gland within 5 minutes after the injection. The longer the fall of blood pressure lasted, the longer the hypersecretion of epinephrin lasted. The level of blood sugar was increased by histamine to a fairly marked degree. When histamine was given after denervation of the suprarenal gland, however, the output of epinephrin was decreased.—Margaret Baptist.

ENDOCRINE GENERAL

ABT. I. A. Endocrines in relation to growth in children. *Illinois M. J.* 77: 137. 1940.

The author discusses heredity, environment, cerebral activity, interglandular relationship, defective nutrition due to infections or metabolic disturbances, vitamin deficiency as well as the various clinical entities characterized essentially by abnormal bodily growth and development.—I. B.

CORYN, G. Influence of endocrine glands on the histology of epiphyseal cartilage (Recherche expérimentale sur l'influence des glandes endocrines sur l'histologie du cartilage de conjugaison). *Ann. d'anat. path.* 16: 27. 1939.

Antuitrine (1 cc. per day for 10 days) did not alter the effects of hypophysectomy on the cartilages in a dog and 30 days treatment of a young rabbit was negative. The effects of castration on the cartilage was partly prevented by injection of 500 U of follicular hormone for 20 days in a rabbit. Negative results were obtained with parathormone (3 rabbits). Thyroxin stimulated growth of the epiphyseal cartilage more or less in proportion to the dose, but this was precociously arrested (2 rabbits).—A. T. R.

GIANTURCO, C. Roentgenological manifestations of endocrine dysfunctions. *Illinois M. J.* 77: 171. 1940.

There are many cases in which roentgenological studies of endocrine diseases are indispensable and of capital importance; in many other cases roentgenological studies may prove to be of great help to supplement the clinical symptoms and support the clinical diagnosis. The author presents illustrative roentgenograms of cases of tumors and dysfunction of the hypophysis, enlargement of the thymus, enlargement and dysfunction of the thyroid, tumors of the parathyroid glands, enlargement and tumors of the adrenal glands and en-

deavors to establish the indications for the roentgenologic examination.—I. B.

HUTTON, J. H., AND M. A. SCHILLER. Endocrine therapy in the psychoses. *Elgin Papers* (*Elgin, Ill. State Hosp.*). 3: 183. 1939.

Of 143 patients referred to the endocrine clinic, 40.5% were cases of dementia praecox. This large number may be due to the fact that about 60% of the hospital population consists of patients with dementia praecox. The presenting symptoms according to their frequency were: menopausal symptoms, obesity, and menstrual irregularities. Secondary ovarian deficiency was the most frequent endocrinopathy, while 28 patients who were referred had no demonstrable endocrine disorder. Of those who received treatment, 7.1% recovered while 31.2% improved. All four cases of myxedema improved, and one case of hyperthyroidism recovered following thyroidectomy. The manic-depressive psychoses and involutional depressions showed the greatest number of improvements, as was to be expected. The involutional psychoses were treated with regular and large doses of theelin or amniotin and improvements occurred in 2 of 6 cases of involutional melancholia. On the other hand, 4 of 8 cases of involutional depression improved. The relation between endocrine dysfunction and psychoses has not been proved or disproved, although there seem to be more obesity, menstrual disorders and other endocrinopathies in the hospital population than in the general population.—*Authors' summary.*

LAKE, G. B. Endocrines and the general practitioner. *Illinois M. J.* 77: 162. 1940.

Endocrine disorders are always pluriglandular. Pure, single hormones, given parenterally, have definite and important uses, but their effects are different from those of whole gland extracts given by injection or by mouth, and cannot replace them in general clinical practice. The commonest endocrine disorder is the climacteric in both sexes, and the well-informed physician can do much to make his middle-aged patients more comfortable and happier. Definite organic disease may coexist with a glandular dysfunction and no amount of enthusiasm for endocrinology should be permitted to betray us into overlooking such cases. All types of infections as well as the stress and wear of modern life tax the adrenals, so that most of the patients need adrenal cortex support at one time or another. The administration of thyroid extracts to all pregnant women would obviate most of the cases of thyroid deficiency in early life and such extracts will frequently give much relief to adults suffering with such deficiencies. Most cases of toxic goiter can be handled satisfactorily without surgery. Every "problem child" is entitled to a thorough and intelligent study of his pituitary and thymus glands, as well as of his domestic environment.—I. B.

RAHMAN, L., H. B. RICHARDSON AND H. S. RIPLEY. Anorexia nervosa with psychiatric observations. *Psychosomatic Medicine* 1: 335. 1939.

Twelve cases are reported in detail. The physical characteristics included emaciation, dry skin, low body temperature and blood pressure and slow pulse. Amenorrhea was present in all cases and in all but one small internal genitalia were found. The vaginal smears were of the atrophic "menopausal" type. In most cases menstruation returned with improved nutrition. The B.M.R. ranged from 60 to 96% of prediction. The findings are regarded as confirming the belief that starvation produces the same effects on metabolism and the endocrine system as does destruction of the anterior pituitary. Treatment with anterior pituitary growth or estrogenic hormone gave no definite benefit and thyroid proved harmful.—R. G. H.

ROUSSY G., AND M. MOSINGER. Correlation between pinealhypophysial interrelations (Les corrélations épiphyso-hypophysaires—Le système neuro-endocrinien du cerveau). *Ann. d' anat. path.* 15: 847. 1938.

A highly theoretical discussion and a complicated diagram of the probable functional relationship between the pineal gland, hypophysis and various brain centers and the sympathetic nervous system—the epiphysis being regarded as antagonistic to the hypophysis.—A. T. R.

SELINGER, E. The ophthalmological aspects of disturbances of the endocrine system. *Illinois M. J.* 77: 167. 1940.

Many eye diseases result from a disturbance or disease of the glands of internal secretion. Some of them, like diabetic retinopathy and proptosis in exophthalmic goiter, are universally recognized to be on that basis. We are likely to forget, however, that others such as iridocyclitis, idiopathic blepharospasm, accommodation insufficiency and many others may also be caused by an endocrine disturbance. An examination of the endocrine system should be made part of the routine physical examination when searching for a possible systemic cause of diseases of the eye such as iridocyclitis, papillitis, retrobulbar neuritis and others. The ophthalmologist should also bear this etiological factor in mind when treating intractable cases of asthenopia, epiphora, blepharospasm, recurrent chalazia and other conditions that persist in spite of the usual medical management. In some of these the endocrine disturbance is the actual cause of the eye disease while in others it throws the reparative defense mechanism out of equilibrium so that the usual medication is of no avail.—I. B.

GONADS

ABARBANEL, A. R. Rationale for the use of testosterone propionate in the immediate treatment of excessive uterine bleeding. *Am. J. Obst. & Gynec.* 39: 243. 1940.

By comparison of numerous observations on varied species, the thesis is presented that testosterone has stimulating effects on both sexes. The phenomena of menstrual bleeding are discussed as primarily under vascular control. From anatomic studies certain functions of myometrial and endometrial arterioles are assumed. Testosterone is believed to reduce bleeding by two effects: by inhibiting rhythmic contractions of myometrium the supply of blood to the endometrium is reduced, and by causing a stimulation of the myometrium, the volume of blood flow in the uterus is further reduced. Clinical trials were made in 25 women of ages 15 to 49, by administration of 10 to 30 mg. doses of testosterone propionate. Some doses were given intramuscularly, others subcutaneously, and some by oral route, combined with bile salts. Results were not tabulated. No important signs of masculinization occurred.—E. L. S.

ABUREL, E., V. D. MARZA AND V. DOBROVICI. Structural malformation of the ovary resembling a tubular adenoma of testicular type (Malformation structurale de l'ovaire ayant l'aspect de l'adènome tubulaire a type testiculaire). *Ann. d' anat. path.* 16: 617. 1939.

A 22-yr. ♀ who had never menstruated, with no axillary or pubic hair and no virilism, and with well developed but painful breasts, subject to headache, dizziness, hot flushes and nervousness, etc., was found to have no uterus but normal appearing ovaries. Biopsy of the ovaries revealed nodules of tissue resembling developing testis or Pick's tubular adenoma, which may be a form of hermaphroditism or an intersexuality brought about by disturbed hormone activity. Five % phenol was injected into ovarian pedicles, uterine ligaments and Müllerian ducts. Ovaries were scarified, bathed with the phenol solution and injected with 1000 U of "glandolobine." A piece of normal ovary was grafted into the abdominal muscles. Relief from most of the symptoms continued during the period of observation (14 months).—A. T. R.

BUKY, F. R. Effect of amniotin therapy in involutional psychoses. *Elgin Papers (Elgin, Ill. State Hosp.).* 3: 179. 1939.

In five cases of involutional psychosis treatment with estrogen proved unavailing.—R. G. H.

CONTAMIN, R., AND F. LERAILLEZ. Clinical use and therapeutic results with gonadotropic hormone (Utilisation clinique et résultats thérapeutiques de l'hormone gonadotrope). *Presse méd.* 47: 1660. 1939.

Extract from PMS and from PU was used without discrimination in 66 ♀ patients. Prompt hemostasis was obtained in cases of uterine bleeding of puberty or of menopause. Excellent results were obtained also in the treatment of nervousness and asthenia, the beneficial effect persisting after cessation of treatment. Encouraging results were observed in treatment of dysmenorrhea, sterility, pruritis vulvae and cutaneous affections associated with menstruation.—A. E. M.

DUYVENÉ DE WIT, J. J., AND V. M. OPPERS. Consideration on removal of corpus luteum verum during early pregnancy and progesterone content of this organ and of mature placenta. *Nederl. Tijdschr. v. geneesk.* 83: 4001. 1939.

Duyvené de Wit and Oppers report a case in which the corpus luteum of pregnancy was removed on the eighty-third day after the last menstruation. From this time until the sixth month of pregnancy progesterone was administered. Unimpaired by occasional uterine contractions, the pregnancy took a normal course. The authors further cite reports from the literature, comprising 131 cases, in which the corpus luteum was removed during the first four months of pregnancy. A review of these cases discloses that in the absence of substitutional therapy abortion results in 23.7 per cent of the cases. With regard to the treatment, the authors take the following position: In ovariectomies during pregnancy, progesterone should be given prophylactically and for some weeks after the operation. If there are no threatening symptoms and a careful supervision is possible, an expectant attitude can be taken, because in three out of four cases the pregnancy will remain intact and take a normal course without substitutional therapy. A clear understanding of the progesterone exchange can be obtained only if it is determined how much progesterone exists at a certain time in the organs preparing it, in the blood, in the organs consuming it and in the products of excretion. In the case reported by the authors, Duyvené de Wit's bitterling test was employed by the determination of the progesterone content. The corpus luteum of pregnancy which was removed from the woman weighed 1.3 gm. and it was found to contain 33 micrograms of progesterone per gram of tissue. This is about twice as much as is found in a mature menstrual corpus luteum. The placenta, which weighed 480 gm., contained 640 micrograms of progesterone. —J.A.M.A.

ENGELHART, E. Evaluation of an orally effective corpus luteum hormone preparation in castrated women (Ueber die Auswertung eines peroral wirksamen Gelbkörperhormonpräparates bei

der kastratierten Frau). *Zentralbl. f. Gynäk.* 64: 98. 1940.

Proluton C (pregneninolon) given orally to rabbits caused the inhibition of uterine motility under posterior pituitary extracts and produced secretory endometrium. Trials in women after priming with 30 mg. doses of estradiol benzoate in oil over 16 days, were based on doses of 25, 50, or 100 mg. daily doses of proluton C. With the two smaller doses flows began before the treatment was stopped, and in currettage specimens there was a slight secretory effect in one case. With the 100 mg. doses, flows followed only after cessation of the proluton C, secretory endometrium was produced, and the success was as good with 5 days as with 8 or 10. The results suggest that it requires 10 to 15 times as much of this material orally as of injected progesterone.—E. L. S.

Escomel, E. The ovary of Lina Medina, the youngest mother of the world (L'ovaire de Lina Medina, la plus jeune mère du monde). *Presse méd.* 47: 1648. 1939.

During the Caesarean operation a biopsy specimen was taken from the ovary. It contained a large number of primordial follicles in evolution, also many atretic follicles. In general the ovary showed the characteristics of full maturity.—A. E. M.

Gardner, W. U., A. Kirschbaum and L. C. Strong. Lymphoid tumors in mice receiving estrogens. *Arch. Path.* 29: 1. 1940.

Estrogens in varying amounts were administered to 149 mice of the CH_3 strain, commencing at ages of 3 to 140 days, in subcutaneous doses of 50γ estradiol dipropionate weekly, 16.6γ estradiol benzoate plus 0.5 mg. carotene, 100γ equilin benzoate plus 0.05 cc. adrenal cortex, and 33.3γ estradiol. Of the mice receiving estrogens 22, or 15.4%, showed lymphoid tumors, in contrast to 0% in 117 untreated mice of the same strain.—L. B. S.

González, A. M. Endocrine treatment in hypertrophy of prostate. *Revista de la Sanidad Militar, Havana* 3: 284. 1939.

Muxó González administered testosterone propionate to a group of twenty-five patients who had hypertrophy of the prostate of either the first, second or third degree. The group included (1) patients with moderate or no retention of urine and a normal amount of urea in the blood, (2) patients with acute retention and a high amount of urea in the blood and (3) patients with grave retention and general intoxication. The substance was administered by injections, every other day, in doses of 0.025 gm. for each injection over a period of three or four weeks. The treatment was repeated after an interval of two months in grave cases at the rate of two injections of the mentioned dose for one week (and in rare grave cases for a month). The author concludes that the testosterone propionate treatment diminishes (or else controls) painful difficult urination and frequent urination. Voluntary micturation becomes free, forceful and abundant. Residual urine, azotemia and hypertrophy diminish (and in some cases disappear). The sexual functions and the general condition of the patient improve. The best results are obtained when the treatment is administered early in the development of hypertrophy. The treatment is also of value for the preparation of patients for a partial endoscopic prostatectomy and as a complementary treatment after prostatectomy. The treatment has its proper indications and it is not a substitute for prostatectomy when the latter is indicated. The author points out the advisability of considering the dose of 0.025 gm. of the substance as the largest to be administered every other day. The administration of a larger dose or of daily doses in the same amount may cause painful erections.—*From J.A.M.A.*

Lauterwein, C. Peroral progesterone therapy (Perorale Progesterontherapie). *Zentralbl. f. Gynäk.* 64: 108. 1940.

Clinical trials were made with "lutocyclin," which is synthetic anhydro-oxy-progesterone. It is suggested in conclusion that the use of a series of 10 daily doses of this substance orally, after appropriate estrogenic stimulation of the endometrium, spontaneously or therapeutically may be expected to produce a secretory endometrium. This applies to secondary amenorrheas, hyperplastic or menopausal bleedings. In treatment of threatened or repeated abortions it is suggested that 5 to 10 mg. doses be given 1 to 3 times daily, well before meals.—E. L. S.

Lipschutz, A., L. Vargas and O. Ruz. Antitumorigenic action of testosterone. *Lancet* 237: 867. 1939.

Three subcutaneous injections of 40 and 80γ estradiol benzoate weekly were given 15 castrated guinea pigs weighing from 360 to 750 gm. Simultaneously varying quantities of testosterone were injected. Proportions of estradiol to testosterone up to 1:13 resulted in development of uterine and extra-uterine fibroids usually resulting from estradiol alone. With a proportion of 1:22 however no fibroids of appreciable size developed, although occasionally some fibrous reaction or tumoral seed appeared. Results were interpreted to indicate that testosterone propionate inhibited the estradiol effect of causing atypical growth of endometrium and its glands.—L. B. S.

Nathanson, I. T., and D. B. Weisberger. The treatment of leukoplakia buccalis and related

lesions with estrogenic hormones. *New England J. M.* 221: 556. 1939.

Leukoplakia buccalis in 25 women and 13 men was treated by the parental administration of estradiol benzoate or the oral administration of α estradiol. The majority of the women received six injections of 10,000 R.U. of estradiol benzoate and 0.5 mg. of α estradiol in tablets by mouth but the men received only the oral therapy. In 16 of the patients the lesions completely disappeared, in 15 there was marked improvement and in only 7 was the treatment without effect. Two of the men developed mastitis, one after 60 days treatment with 0.17 mg. daily and the other after 90 days on 0.5 mg. daily. It is suggested that disordered hormone metabolism leads not only to disturbances of the mucosa of the genital tract but also to lesions of the mouth and nose.—J. M. L.

Reist, A. Protective action of large doses of corpus luteum on pregnancy in operations on the pregnant uterus or neighboring organs (Die protektive Wirkung hoher Corpus-luteum-Dosen auf die Schwangerschaft bei Operationen am schwangeren Uterus oder seinen Nachbarorganen.) *Zentralbl. f. Gynäk.* 64: 103. 1940.

In operating on 5 women with uterine or pelvic operations the author thinks the stability of the pregnancy was enhanced by the use of 5 to 10 mg. doses of progesterone before surgery, for 1 to 3 days, and for 5 or more days afterward. Total doses were 38 to 58 mg.—E. L. S.

Ripley, H. S., E. Shorr and G. N. Papanicolaou. The effect of treatment of depression in the menopause with estrogenic hormone. *Am. J. Psychiatry* 96: 905. 1940.

Twelve patients showing depressions of 3 technical clinical types were treated with progynon-B or amniotin in dosages sufficient to influence the vaginal smears. The total dosages ranged from 48,000 to 851,000 R.U. per patient. Five of the 12 patients showed no improvement and only 1, marked improvement. The amelioration was confined to vasomotor symptoms and feelings of well-being; no evidence was seen that the illness, as such, was specifically influenced or shortened in its course.—R. G. H.

Sharpley-Schafer, E. P., and I. Schrire. Effect of oestrogens on the urinary volume. *Lancet* 237: 973. 1939.

Fourteen ♂ and ♀ subjects ranging from 16 to 61 years of age were studied at bed rest and under uniform conditions. Each patient was put on a suitable fixed diet of known salt and water content. Twenty-four hour samples of urine were measured. Estrogens in the form of estradiol benzoate, 100,000 I.B.U. were injected once daily intramuscularly over a period of 10 days. No change could be detected in urinary volume, either in normal, menopausal or castrate individuals.—L. B. S.

Silberman, D., H. M. Radman and A. R. Abarbanel. The use of testosterone propionate in the treatment of the menopausal patient. *Am. J. Obst. & Gynec.* 39: 332. 1940.

The treatment of 15 women with menopausal symptoms was carried out by injecting subcutaneously 5 mg. of testosterone propionate in oil 2 or 3 times weekly, by oral administration of 5 to 10 mg. doses, or by implantation of 25 mg. as pellets subcutaneously. The latter was the favored method. Barbiturates as sedatives were also used frequently. Results were considered satisfactory, although the few case histories summarized do not mention complete relief from symptoms. Three women had "tanning" of the skin, as observed in males treated with testosterone.—E. L. S.

Spence, A. W. Testosterone propionate in chronic mastitis. *Lancet* 237: 820. 1939.

Forty patients aged 19 to 52 years were studied. In 24, sterile olive oil injections were given intramuscularly, with relief of pain in 13. Sixteen patients were treated with testosterone propionate, intramuscularly in doses of 25, 50, 100 mg. twice weekly for several months. In 5 patients lumps in the breasts disappeared, although in 2 the possibility of spontaneous disappearance could not be excluded. In 1 patient, given 2925 mg. over a 5-month period, hypertrophy of the clitoris resulted, together with extreme atrophy of the endometrium. In 5 patients reduction occurred in size of breast nodules, and in 2 fresh nodules appeared during the course of treatment. Menstruation was suppressed in 7. Increased hair growth occurred in 5 younger patients; this was not observed in older individuals receiving larger doses. Caution is urged in the use of testosterone propionate.—L. B. S.

HYPOPHYSIS

Baranao, A. C. Thyrostimulin (Tireostimulina). *Semana méd. Buenos Aires* 47: 1. 1940.

Thyrotropic pituitary extract was prepared by Collip's method. It produced in immature guinea pigs a temporary stimulation of the thyroid which regressed as treatment was continued, resulting in a gland of normal histological characteristics. No changes were observed in the sex organs. Colloid in the glands of infantile animals disappeared and that in adults was considerably reduced. Simultaneous injection of thyroid globulin led to almost complete inhibition of the effect. Thyrostimulin caused an increase in the B.M.R. in mice. Simultaneous administration of quantities of each which, separately, would increase B.M.R. by 7%, induced no metabolic change. Thyroid hormone

caused increased contractions of an intestinal loop by parasympathetic action, whereas thyrostimulin caused relaxation through sympathetic mediation. It had no effect on uterine contractility, on the sex cycle, nor on arterial pressure. It was concluded that the pituitary hormone is not an activator of the thyroid but causes a release of hormone contained in the gland, and that it is antagonistic to the thyroid hormone.—A. E. M.

COLLIP, J. B. The physiology of the anterior pituitary and a note on the medullotropic hormone. *Am. J. Obst. & Gynec.* 39: 187. 1940.

Following a conservative review of the physiology of various pituitary extracts, with mention of methods of preparation, there is preliminary description of the effect of injecting the "specific metabolic extract" into chicks, as a consequence of which depôt fat is decolorized and blood and liver vitamin A is altered. This same extract in women has shown an unexpected ability to reduce irregularity of intermenstrual intervals, but without significant help in control of obesity. There is evidence that an alcoholic extract of pituitary glands can be administered orally, with stimulation of hypertrophy of the "dark cells" of the adrenal medulla.—E. L. S.

COSTERO, I., AND H. BERDET. Tumors of the hypophysis and hypophysial duct (Estudio anatómico de 135 tumores de la hipófisis y de tracto hipofisario). *Monogr. d. l. Soc. Med. d. Hosp. Gen., Mexico* No. 1: 1–116. 1939.

One hundred thirty-five tumors of the hypophysis were described histopathologically and classified in unusual detail. The relationship between their structure, symptomatology and prognosis was considered. Three types of chromophobe adenoma were recognized, namely, adenocystoma, adenoepithelioma and adenocarcinoma. Chromophil adenoma were subdivided into oligochrome (exhausted acidophil, mixed acidophil and chromophobic, pregnancy cell, transitional and others), acidophil proper, basophil and large-cell (frustrated acidophil cell) adenoma. No tumors were derived from the pituicytes (neuroglia cells) of the neurohypophysis. Chromophobe adenomas did not produce hormones but may produce colloid. The close relation of acidophil tumors to acromegaly was reaffirmed. The role of basophilic granulation to Cushing's syndrome is not considered established. An extensive bibliography is included.—A. T. R.

HERRE, W., AND F. RAUVIEL. Implantation of additional hypophysis into different species of salamander larvae (Implantation zusätzlicher Hypophysen in Larven verschiedener Molcharten). *Arch. f. Anat. u. Entwcklngsmechn. d. Organ.* 139: 86. 1939.

Implantation of the hypophysis of adult salamanders into larvae in general caused hypertrophy of the sex organs. In male triturus cristatus danubialis there was pronounced swelling of the cloaca and transformation of its border from the larval condition to a nuptial crest. The testes were enlarged and contained spermatozoa. In females of this species no difference was obvious in the ovary; but in Triturus marmoratus there was an increase in the size and development of the ovary. Both sexes responded in Triturus helveticus. Growth and metamorphosis apparently are not related to the development of the germ cells.—A. T. R.

KENNY, M., AND E. KING. Effect of prolactin on lactation in nursing women. *Lancet* 237: 828. 1939.

Preparations of prolactin from ox and from sheep pituitaries were administered in 43 cases of secretory deficiency at different stages of lactation in doses of 15 cc. (900 Riddle u.) spread over 5 days, intramuscularly. The extracts appeared to have no local or systemic effects. Glucose tolerance tests were negative, and gonadotropic function apparently was unimpaired. The quality of milk secreted was essentially the same as that of control samples.—L. B. S.

MEYER, A. Histological studies on normal and operatively altered hypophysis of Triton vulgaris (Histologische Untersuchungyn an normalen und operativ veränderten Triton vulgaris-Hypophysen). *Arch. f. Anat. u. Entwcklngsmeckn. d. Organ.* 139: 209. 1939.

Pars tuberalis, unlike that of most urodeles and similar to that of anurans, becomes separated from the ventral lobe during development. Basophils and acidophils differentiate first and not until after metamorphosis do typical chromophobes appear. In adults the author divides the ventral (anterior) lobe into four different regions on the basis of the relative number of the different types of cells. Partial and complete extirpation of the hypophysis was carried out on many larvae of 20–23 mm. length. About 1/9 of the normal anterior lobe is sufficient for metamorphosis. Remnants of pars intermedia produce dark larvae. Damage to the floor of the diencephalon in animals with intact hypophysis produces darkened animals with delayed metamorphosis.—A. T. R.

SEVERINGHAUS, A. E., AND K. W. THOMPSON. Cytological changes induced in the hypophysis by the prolonged administration of pituitary extract. *Am. J. Path.* 15: 391. 1939.

A cytological study has been made of the anterior lobe of the hypophyses of dogs and sheep after prolonged administration of extracts of sheep pituitary gland (van Dyke and Wallen-Lawrence). Thompson had previously reported the atrophy of the thyroid, adrenals and gonads in these animals, and a syndrome which resembles that of pituitary basophilism in man. The anterior lobe is

markedly altered. The basophiles show extreme modification. While normally granulated basophiles may be found by search, most of the cells have become greatly hypertrophied, modified through vacuolation of several varieties, and by coarse clumping of the basophilic granules. All stages in the vacuolation and hyalinization phenomena can be readily traced. Modified basophiles resemble those seen after castration, thyroidectomy, or in human pituitary basophilism. In addition, many of the cells can be seen in process of complete disintegration. Evidence is advanced to support the contention that these vacuolar phenomena are atrophic in character. Areas of the gland show basophiles rich in mitochondria and in stages of degranulation, which are interpreted as cytological evidence of secretory activity. The acidophiles appear essentially normal, showing the modifications which are characteristic of their cycle of secretory activity. The glands are richly vascular and in addition contain numerous edematous areas. The hypophyses of ewes injected with an extract of sheep pituitary gland showed a marked degranulation of basophiles, while the acidophiles appeared more compact and brilliantly strained. Cytological evidence of increased secretory activity is present. Dogs injected with anti-hormone dog serum show an edematous and hyperemic anterior lobe. Basophiles show extensive vacuolation and may appear strikingly similar to the hyalinized cells of the Cushing syndrome of pituitary basophilism. An interpretation of the phenomena is suggested.—*Abstract, J. Nerv. & Ment. Dis.*

PANCREAS

Bennett, C. R., and T. K. Miller. An observation of the treatment of mental cases with subshock doses of insulin. *Am. J. Psychiat.* 96. 951. 1940.

Insulin was given in dosage of 15 to 50 u twice daily to 125 patients who presented ward-behavior problems. In nearly every case acute maniac excitement was promptly controlled. The use of sedation was reduced to 60% in general and in extreme psychomotor activity almost entirely. Tube feeding was eliminated in 90% of the cases. Early clinical improvement in a considerable proportion of cases of major psychoses was seen. The weight increased on the average 15 pounds. Carbuncles, boils and sometimes acne, when present, promptly disappeared. The necessity for a "disturbed ward" was practically eliminated.—R. G. H.

Erspamer, V. The relation of the enterochromafin system to the islands of Langerhans (Il sistema enterocromaffine ed i suoi rapporti con il sistema insulare). *Ztschr. f. Anat. u. Entwcklngsgesch.* 109: 587. 1939.

The nature, histogenesis and topographical distribution of certain cells with an affinity for silver (found in the pancreas and adjacent organs) and their status as pre-insular cells are discussed.—A. T. R.

Greenler, D. P. Pancreatic islet tumors with hypglycemia. *Pennsylvania M. J.* 43: 809. 1940.

This paper consists of a brief discussion of the subject with 2 illustrative case reports. In disturbed states of consciousness where a definite diagnosis cannot be established, routine blood chemistry determinations should be made to establish whether hypoglycemia is the cause of the disorder. Where such a condition has been found to be present and the cause found to be due to a pancreatic islet cell tumor, surgical removal has produced cures in the majority of cases.—I. B.

Grishaw, W. H., H. F. West and B. Smith Juvenile diabetes mellitus. *Arch. Int. Med.* 64: 787. 1939.

This report is a review of certain statistical findings on juvenile diabetes mellitus for comparison with other reports. There were 341 children in the group, all with diabetes observed between April 1920 and January 1938 except 2 seen in 1917. In all of them diabetes developed before the 16th birthday, the earliest onset being at the age of 8 weeks and only 4 children being under 1 year of age when the disease was discovered. The highest incidence peak for the entire group occurred at the 11 to 12 year period but there was another high peak at the age of 7 to 8 years. The peaks for girls occurred at 3 to 4 and 7 to 8 years, with a constant high level at 9 to 10, 10 to 11 and 11 to 12 years, while for boys 7 to 8 and 11 to 12 were the outstanding ages. The sharp drop in incidence of onset during the fifteenth year seems to be universally observed. No new theories or principles were developed in the course of the study.—I. B.

Helwig, E. B. Hypertrophy and hyperplasia of islands of Langerhans in infants born of diabetic mothers. *Arch. Int. Med.* 65: 221. 1940.

The pancreases of 9 infants of diabetic mothers and of 9 infants of nondiabetic mothers were studied. The islands of Langerhans in the former exhibited a variable degree of hypertrophy and hyperplasia, most marked in those infants nearing full term when they were born. The nuclei were frequently enlarged and occasionally hyperchromatic. The stroma and to a less degree the islands of the infants of diabetic mothers frequently were infiltrated with eosinophilic granulocytes, many of which were mature. This was not present in the control group. One infant in the control group, whose mother was not known to have diabetes, showed hypertrophy and hyperplasia of the islands and no obvious anatomic cause of death. It is concluded that some infants of diabetic mothers have definitely larger amounts of insular tissue than infants of nondiabetic mothers. This is marked in

those infants whose greater birth weights suggest a possible excess growth stimulus. On the other hand, it must be recognized that a fair number of the pancreases of infants of diabetic mothers will fall in the normal range of insular size. The determinations of blood sugar on the whole show little correlation with insular size, the lowest level of blood sugar, 30 mg. per hundred cc. occurring in an infant dying of subtentorial hemorrhage, and one of the higher values, 190 mg. per hundred cc., occurring in a stillbnor infant who had the largest islands of any in the series.—I. B.

MORRIS, N. Newer insulins. *Glasgow M. J.* 15: 1. 1940.

This is a historical review of the treatment of diabetes and of insulin, leading up to the current usage of protamine zinc insulin. There are many advantages from the use of protamine zinc insulin, the most important of which is the abolition of states of transient ketonemia resulting from tissue breakdown. Moreover, patients admit of an improved sense of well-being from protamine zinc insulin. Liver metabolism is likewise favored. The threat of coma is reduced. These advantages are more clearly observed in adults than in children. Protamine zinc insulin is contraindicated in diarrhea and hypothyroidism.—I. B.

YOUNG, F. G. The anterior pituitary gland and diabetes mellitus. *New England J. M.* 221: 635. 1939.

This article summarizes present knowledge of the relationship between the anterior pituitary gland and diabetes mellitus. In certain cases dogs injected with extracts of the anterior pituitary gland became permanently diabetic and their islets of Langerhans were grossly abnormal, but in other animals the islets changes were not sufficient to account for the severity of the diabetes. The amount of insulin required to regulate the glycosuria of the pituitary diabetic dog was significantly greater than that required for the depancreatized dog consuming the same amount of food. Despite this they were able to survive for longer periods when insulin was not administered. Treatment with anterior pituitary extract produces a resistance to insulin which may have an extra pancreatic factor and may be the explanation for those human diabetic patients that require extremely high amounts of insulin (1000 U or more) daily to control the disease.—J. M. L.

PARATHYROID

CARRIÈRE, G., AND A. VERHAEGHE. The parathyroids in tuberculosis of adults (Les parathyroïdes dans la tuberculose de l'adulte). *Ann. d' anat. path.* 16: 407. 1939.

In 270 autopsies at Saint-Sauveur Hospital (Lille), 60 showed signs of tuberculosis (28 of whom certainly died of this disease) but only 3 had tuberculous lesion in the parathyroids. The pathology is described. Parathyroid tetany is extremely rare in tuberculosis.—A. T. R.

CHANG, C., AND T. CHEN. Parathyroid and hypocalcemia in vegetarian rats. *Chinese J. Physiol.* 15: 19. 1940.

Parathyroid glands and blood Ca of normal rats, 11 of each sex, were compared with those of rats fed a special vegetarian diet, 33 ♂♂ and 25 ♀♀. The glands of the experimental rats weighed 1.5 to 11.0 mg. each, those of the controls 0.2 to 0.6 mg. Blood Ca of the vegetarian animals averaged 20 to 30% lower than the normal value of 9.9 to 11.2 mgm. per 100 cc. of serum. The authors believe the abnormalities were probably due to vitamin D deficiency, although tabulation shows an average Ca intake of the vegetarians of about 300 mg. per week, compared with 1096 mg. per week for the controls. The Ca/P ratio was also lower for the vegetarians, averaging 0.73 as compared with 1.38 for the controls.—R. L. Z.

THYROID

ALTHAUSEN, T. L., J. C. LOCKHART AND M. H. SOLEY. A new diagnostic test (galactose) for thyroid disease. *Am. J. M. Sc.* 199. 342. 1940.

The galactose is given in 40 gm. doses by mouth. Venous blood for analysis is obtained after 30 and 60 minutes. The non-fermentable reducing material of the specimen is analyzed as glucose and the conversion to galactose value made by adding 24%. It was found that 130 patients with clinical hyperthyroidism gave a maximal blood-galactose concentration three times higher than that of 121 control subjects. In reliability the test compared favorably with B.M.R. determinations. Following thyroidectomy, the galactose tolerance test was normal in almost all cases. The test proved to be more sensitive than the basal metabolic rate in cases of low grade hyperthyroidism, and its outcome was not influenced by hyperventilation in anxiety states or by cardiac dyspnea. A disadvantage of the test is that the presence of hepatic insufficiency or of Paget's disease interferes with its use for the diagnosis of thyroid disease. In myxedema abnormally low galactose tolerance curves were observed, indicating that the galactose test can be used also in the diagnosis of this condition.—R. G. H.

BRAIN, W. R. Exophthalmos in Graves's disease despite sympathetic paralysis. *Lancet* 237: 1217. 1939.

A married woman aged 31, suffering from paralysis of the ocular sumpathetic as a result of syringomyelia, developed Grave's disease, showing both exophthalmos and a retraction of the upper lids. It is concluded that ocular abnormalities are not produced by overactivity of the sympathetic.—L. B. S.

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